BIOLOGICAL SCIENCES DIVISON

BSD-1

A SITUATIONER ON MICROALGAE AS ALTERNATIVE SOURCE FOR BIOFUEL

Nina M. Cadiz[1*], Qiang Hu[2] and Milton Sommerfeld[2]

[1]Institute of Biological Sciences
University of the Philippines Los Baños, College, Laguna 4031 Philippines
*E-mail: nmcadiz@uplb.edu.ph
[2]Department of Applied Biological Sciences
Arizona State University, Mesa, AZ 85212, U.S.A.

The escalating price of petroleum and the rising concern about global warming due to the burning of fossil fuel has encouraged plant biologists, engineers and other scientists to look for alternative sources of energy. At present, plant (e.g. soybean, corn) and animal fats still serve as a major non-petroleum source of biodiesel. The concept of using microalgae as an alternative source of renewable energy, is becoming popular because of their high photosynthetic efficiency and capacity to accumulate large amount of natural oils in their cells. Microalgae can provide several types of renewable biofuels, including methane, ethanol, biodiesel and biohydrogen, or can be processed to make biocrude, a renewable equivalent of petroleum. The oil yield per acre from microalgae is far greater than soybeans or corn. Microalgae can be grown in either open ponds or photobioreactors and require much less land area and water for equivalent oil production.

Some promising species of microalgae for biofuel production are the green algae *Botryococcus braunii*, *Chorella sp.*, *Nannochloropsis*, and the diatom, *Nitzschia* sp. which we also have in the Philippines. There is increasing interest in exploring and exploiting the use of algae for biofuel production, with the number of companies publicly announcing their interest in microalgae biofuels increasing from less than five three years ago to over fifty today. The companies are forging ties with universities, national laboratories and governmental agencies to develop and commercialize the microalgae-based biofuel production technologies. These efforts have been initiated in several countries, indicating a broad and international interest in microalgae-based renewable biofuels. In the Philippines, particularly the University of the Philippines Los Baños, research efforts have initially been started. Our project proposal on the potential of microalgae for biodiesel has long been submitted for funding.

Keywords: microalgae, renewable biofuels, methane, biohydrogen, biodiesel, feedstocks

BSD-2

SWEET SORGHUM FOR BIOFUEL, FOOD, FEED AND FORAGE

Samuel .S. Franco*, Heraldo L. Layaoen, Mario I. Remulacio, Thelma .Z. Layaoen, Prima Fe. R. Franco, Lorceli B. Taclan, Ana Fe C. Llaguno, Vina A. Cabugon, Gary D. Ponferada, Val R. Gudoy, Rose G. Ramos and Odilon.A. Caraan

Mariano Marcos State University
City of Batac, 2906, Ilocos Norte
Telefax: (077) 792-3191
*samuelsfranco@yahoo.com

The utilization of sweet sorghum (Sorghum bicolor (L.) Moench] for bioethanol has been undertaken by the Mariano Marcos State University. Sweet sorghum has been found to be better than other crops because it is the only crop that provides grain and stem that can be used for sugar, alcohol, syrup, jaggery, fodder, fuel, bedding, roofing, fencing, paper and chewing. It is a crop that can be grown and ratooned to give higher annual yields than sugarcane.

Results of the experiments indicate that two varieties, SPV-422 and NTJ-2 have been found to be adaptable under Philippine conditions. These varieties are now being planted and tested in different parts of the country by the Department of Agriculture, State Colleges and Universities and the private sectors. The sorghum grains were also found to be a substitute for flour in making cakes and confectionary. It can also be used as feeds. The bagasse can be used as forage because it is soft.

The sweet sorghum varieties identified can be easily milled utilizing existing farm level sugarcane press. The varieties have a sugar content of 19 to 24% percent which is higher than sugarcane. The juice was found to be easily made to syrup and jaggery. The juice and the jaggery can be fermented by different strains of *Saccharomyces cerevisiae* and *Zymomonas mobilis* and results showed that ethanol produced is up to 12% by volume depending upon the starting amount of feedstock used. Sweet sorghum can be a very good feedstock for bioethanol production and is a very promising crop that could be tapped as an alternative for fossil fuels.

Keywords: alcohol, bioethanol, feed, fermentation, food, forage, jaggery, *Saccharomyces cerevisiae*, sweet sorghum, and *Zymomonas mobilis*

BSD-3

ISOLATION OF MICROORGANISMS FOR FIRST AND SECOND GENERATION ETHANOL PRODUCTION FROM SWEET SORGHUM (*Sorghum bicolor* L.)

Prima Fe R. Franco*, Cecil Gaoat, Leo Garvida, Desiree Dagdagan, Rowena Acosta, Ellen Castillo, Maingeline Bacarisa, Mary Grace Balintag, Samuel . S. Franco and Heraldo L Layaoen

Mariano Marcos State University, City of Batac
*E-mail: primarfranco@gmail.com

There is a worldwide race in the search for alternative sources of energy because of the soaring prices and the dwindling supply of fossil fuels. Biofuels like bioethanol are potential alternatives. Sweet sorghum has a high sugar content in its stalk could be used as feedstock for bioethanol production. Studies on the utilization of sweet sorghum as feedstock for bioethanol production at the laboratory scale using *Saccharomyces cerevisiae* show that the juice and jaggery can be very good fermentable substrates The percent alcohol produced depends on the amount of fermentable sugar and the efficiency of the strain used.. Since the strains used were isolated and optimized for sugarcane as feedstock, the need to scout for ideal fermenters for sweet sorghum as feedstock is deemed necessary. Moreover, previous fermentation studies in the laboratory reveal that the uninoculated feedstock also yielded ethanol which indicate that some native thermotolerant strains must be present. This study isolated, characterized, cultured, and screened fermenters from sweet sorghum juice and jaggery for first generation ethanol production. Results show that ten native isolates were obtained and produced 6.5 to 12% ethanol. Moreover, since the bagasse is still a viable source of second generation ethanol, biodegraders from potential sources like cow cud, cornick wastes and from landfills were isolated and used to degrade sweet sorghum bagasse. Twelve putative cellulose degrading isolates were obtained from the various sources. Initial second generation ethanol from sweet sorghum can be readily achieved using the isolates. All of the isolates from this study have a crucial role in bioethanol production using sweet sorghum as feed stock.

Keywords: biofuels, *Saccraomyces cerevisiae*, fermenters, bioethanol

BSD-4

PERFORMANCE OF JATROPHA SEEDLINGS OUTPLANTED IN ABANDONED MINE SITE OF MOGPOG, MARINDUQUE

Nelly S. Aggangan[1], Nina M. Cadiz[2]*, Nelson M Pampolina[3], Asuncion K Raymundo[2], Lorie C. Trinidad[1] and Veronica P. Migo[1]

[1]National Institute of Biotechnology and Applied Microbiology
[2]Institute of Biological Sciences, College of Arts and Sciences
[3]Department of Forest Biological Sciences,
College of Forestry and Natural Resources,
University of the Philippines Los Baños, Laguna 4031
*Email: nmcadiz@uplb.edu.ph,

Jatropha curcas (physique nut) is a shrub or small tree that is widely distributed in the tropics, including the Philippines. It is a versatile crop whose importance range from its medicinal value, source of biodiesel, organic fertilizer and as feedstocks. It also adapts well in a wide range of stressed condition, like heavy metal-rich soil. For bioremediation of an abandoned mine site in Mogpog Marinduque using Jatropha, the plant's growth was enhanced using different ameliorating treatments. The use of commercial and non commercial endomycorrhizal fungi, together with lime, compost and their combinations were evaluated. The field experiment was laid following randomized complete block design with four blocks and ten seedlings in a row per treatment.

Initial results showed that Jatropha seedlings with no compost and with or without lime exhibited the poorest growth. The tallest and biggest stem diameter were observed in seedlings treated with Mykovam or MineVAM plus compost and lime. Without compost or lime, mycorrhizal inoculation was ineffective. Addition of lime, however, significantly increased stem diameter, root, leaf, stem and total dry weights by 40%, 97%, 42%, 262% and 50%, respectively, as compared with the unlimed mine soil. On the other hand, analysis of heavy metal (Cu, Zn and Pb) translocation in Jatropha, irrespective of the amendments applied, showed a greatly reduced translocation of these heavy metals (HMs) to the stems and leaves. In the control, Zn, Cu and Pb were found at highest concentration in the roots, stems and leaves, respectively. We are awaiting the fruiting stage of the other Jatropha plants since information of HM translocation in the fruits will have a bearing in the use of this plant as a source of biofuel.

Keywords: Jatropha, bioremediation, abandoned mine site, mycorrhiza, heavy metals, translocation

BSD-5

POLLINATION BIOLOGY OF THE PHYSIC NUT, *JATROPHA CURCAS* L.

Aimee Lynn A. Barrion-Dupo

Environmental Biology Division, Institute of Biological Sciences and Museum of Natural History
University of the Philippines Los Baños, 4031 College, Laguna
Telephone: +63 049 536-2893 or 536-3368
Telefax: +63 049 536-2517; Email: aranea95@yahoo.com

Floral biology, pollination and effects of pollination mechanisms on Philippine-cultivated physic nut, *Jatropha curcas* were studied. Results showed that physic nut flowers are monoecious and unisexual. Inflorescences are characteristically composed of many to several green bell-shaped cymes. Male flowers produce around 1800 ± 45 thick-exined inaperturate pollen with 71% viability. In addition, male flowers have 10 stamens, 5 united at the base only, 5 united into a column. On the other hand, female flowers are borne singly.

Floral anthesis was recorded at 4:30 am. *Calliphora* sp., *Apis mellifera*, *Vespa* sp., a pierid butterfly and two species of small ants were observed to pollinate the flowers. Calliphorid flies were observed to be the first pollinators to visit the flowers. They were seen as early as 6:00 am. By 7:00 a.m., two species of small ants (one red and one black) were observed to also pollinate the flowers. This was followed by *Apis mellifera* at 8:00 to 9:00 a.m. Larger red ants arrived around 10:00 a.m. but were only observed as collecting nectar. Wasps and butterflies were only observed only until noon. Other arthropod visitors of *Jatropha* included the ricaniids and certain gargantuan lady beetles.

Meanwhile, flowers from two-year old physic nut plants from Calauan, Laguna were open-pollinated, bagged and hand-pollinated to determine effects of pollination mechanisms. Fruit set was observed a few days to a week after set-up. Both open-and hand-pollinated flowers had exhibited fruit set. However, hand-pollinated plants had produced fewer and smaller fruits than open-pollinated ones. This could have been affected by the amount of pollen transferred with a brush. On the other hand, bagged flowers had no fruit set. Thus, the physic nut, is a cross-pollinated plant.

Keywords: anthesis, cyme, exine, floral biology, floral visitor, physic nut, pollination

BSD-6

MORHO-ANATOMICAL ASSESSMENT OF OIL CELLS IN SEEDS OF *PONGAMIA PINNATA* Merr. (L)

Vivian S. Tolentino[1], Wendel T. Marcelo, Emmanuel M. Espineda, Vivian A. Panes and Evelyn E. De Guzman

Department of Biology, Ateneo de Manila University
Loyola Schools, Katipunan Road, Quezon City
[1]vtolentino2001@yahoo.com

Pongamia pinnata Merr (L), commonly called (Bani) is reported to contain oils in the seeds which may be used as an alternative source of energy. This study aims to describe the structural features of the oil cells and its development. Simple microtechnique procedures like free-hand sectioning of the seed cotyledon was utilized, and different tests for the presence of oils was employed. Characterization and documentation was done using light microscopy. Histochemical tests showed the presence of neutral lipids occurring as single large deposit in idioblastic cell, and as numerous smaller individual droplets within the cytoplasm of parenchyma cells. Idioblastic oil cells measures from 65-75 μm and are slightly larger than the surrounding ordinary parenchyma cells which have varying sizes from 20-70 μm. The lipids in idioblastic cells stain in different density than those in parenchyma cells. They contain a characteristic oil sac within the cytoplasm that encloses the deposited oil. The presence of a cupule was also observed which is another defining characteristic of oil cell. Different stages of oil cell development were also observed which were marked by the sizes of oil accumulation. There is a direct relationship between the amount of oil accumulation and the developmental stage of idioblastic oil cells. As the oil cell matures, the oil deposit also increases in amount until it filled the entire oil sac at maturity. This study can be used as a guide in identifying the location and peculiar features of oil cells which may be cost efficient in terms of extraction and production of oils from plants.

Keywords: oil cells, cupule, idioblast, lipids

BSD-7

IN SILICO ANALYSIS OF CANDIDATE DROUGHT TOLERANT GENES OF RICE DEDUCED FROM GENETIC LINKAGE MAPS

Orlex B. Yllano[1,2*], Richard Tambalo[2], Namrata Singh[2] and Lotise D. Escobin[2]

[1]Graduate Biology Program and University Research Center
Adventist University of the Philippines, Puting Kahoy, Silang, Cavite
[2]Molecular Biology and Biotechnology Program
University of the Philippines Los Baños, College, Laguna
Tel.: 049-5411211; Fax: 049-5411228; *Email: lex@aup.edu.ph;

Cell membrane stability (CMS) is one of the essential traits that was identified and associated with drought tolerance. Cloning of genes linked with CMS is essential in developing drought-tolerant rice varieties in the near future. In this study, *in silico* analysis was utilized to identify the candidate genes for CMS. Using the QTL map (QTL QCMS8.2) previously identified by Tripathy et al. (2000) and using the Gramene database, initial analysis revealed that the two markers (RG598 and EM18) associated with CMS lie on chromosome 8. Sequence search analysis revealed that one of the markers was positioned in APO5251 Bacterial Artificial Chromosome (BAC) contig. Using the Rice AGI FPC (2002), the RG598 marker was determined in the BAC clone of *Oryza sativa* (japonica cultivar group) at 130,146 bases. Using the Basic Local Alignment Search Tool (BLAST), it was determined that the target sequences aligned well with permeases and integral membrane transporters with bit scores of 113 and 100 and with E-value of 6e-24 and 8e-20, respectively. Amino acid and nucleic acid sequences of the candidate genes were downloaded from the Genebank and primers with 20 bases length, 59-60 °C melting temperature (Tm) and guanine-cytosine (GC) content ranging from 45-50% were designed.

Keywords: drought- tolerant genes, *in silico analysis*, cell membrane stability, linkage maps, rice

BSD-8

MARKER-AIDED TRANSFER OF ß-CAROTENE BIOSYNTHETIC GENES INTO POPULAR PHILIPPINE RICE VARIETIES

Antonio A. Alfonso[1], Christine Jade A. Dilla[1], Gerald B. Ravelo[1], Emilie O. Espejo[1], Nelson S. Garcia[1], Eleanor S. Avellanoza[1], Rhodora R. Aldemita[2] and Leocadio S. Sebastian[1]

[1]Plant Breeding and Biotechnology Division
Philippine Rice Research Institute
Maligaya, Science City of Muñoz, Nueva Ecija
[2]International Service for the Acquisition of Agri-Biotech Applications
IRRI Compound, College, Laguna

The development of Golden Rice, a genetically engineered rice variety capable of producing pro-vitamin A carotenoid (ß-carotene) in the endosperm, is envisaged to combat the prevalent problem of Vitamin A deficiency (VAD) in many rice-eating countries. A two-pronged approach was undertaken to develop locally-adapted varieties biofortified with ß-carotene using US rice variety Cocodrie containing event GR309 as donor. The first approach involved the transfer of the Golden Rice trait into two popular varieties (PSB Rc82 and NSIC Rc128) through DNA marker-aided backcrossing. Advanced backcross progenies were produced and evaluated using 60 microsatellite markers distributed in the entire genome. Genetic recovery of the selected third backcross progenies ranged from 80-89%. Based on the presence of GR309 locus as determined using event-specific DNA marker (foreground selection), genetic similarity to the recurrent parent (background selection), overall phenotypic acceptability, and intensity of yellow pigmentation in the grains, five BC3F1 populations from GR309 x NSIC Rc128 and 12 BC3F1 populations from GR309 x PSB Rc82 were advanced to BC3F2. The other approach was to develop new Golden Rice varieties with resistance to tungro and bacterial blight incorporated through conventional breeding. The addition of other important agronomic traits is hoped to facilitate wider adoption by farmers. IR64-derived lines highly resistant to tungro and bacterial blight were used as recurrent parents. Advanced backcross progenies possessing the Golden Rice genes and exhibiting resistance to tungro and bacterial blight have been identified. Production of stable lines carrying the three desired traits is being carried out through self-pollination and anther culture.

Keywords: Golden Rice, vitamin A deficiency, betacarotene, marker-aided backcrossing

BSD-9

DEVELOPMENT OF A MOLECULAR MARKER-BASED PROTOCOL FOR SEED PURITY ANALYSIS IN HYBRID RICE

Loida M. Perez*, Haizel M. Pastor, Teodora E. Mananghaya, Virginia P. Luciano, Dindo A. Tabanao and Norvie L. Manigbas

Plant Breeding and Biotechnology Division
Philippine Rice Research Institute (PhilRice)
Maligaya, Science City of Muñoz, Nueva Ecija, 3119 Philippines
Tel: +63 44 456 0285 loc. 260); Email: *lmperez@philrice.gov.ph, lmoreno_perez@yahoo.com

Seed purity is a major factor contributing to the attainment of higher yield in hybrid varieties in farmers' fields. However, morphological features of commercial rice seeds including inbreds and hybrid rices produced by seed growers and private companies are almost similar. This study aims to develop a molecular protocol in detecting true hybrid type in a seed lot. We have evaluated 302 microsatellite DNA markers in denaturing polyacrylamide gel electrophoresis (PAGE) to determine specific DNA pattern diagnostic to Mestizo 1 (M1), Mestizo 3 (M3) and Mestizo 7 (M7) hybrids. Genomic DNA was extracted using the modified mini scale CTAB extraction method, and polymerase chain reaction of DNA templates was carried out using a conventional thermal cycler machine. RM263 was found to be diagnostic to M3 hybrid samples while RM110 detected the DNA pattern diagnostic to both M1 and M7 hybrid samples. Alleles of RM263 could only be detected in PAGE while alleles of RM110 could be detected in non-denaturing PAGE suitable for high throughput analysis. RM21 and RM190 detected a highly specific DNA pattern diagnostic to M1. Other microsatellite loci such as RM408 and RM588 detected unique alleles for BIO401 and SL8, two of the current hybrid rice varieties in farmers' fields developed by private seed companies. These findings are currently being optimized in a controlled experiment to determine their efficiency and accuracy in detecting contaminants or non-hybrid seeds. The use of molecular techniques involving assessment of seed purity based on DNA fingerprinting is a promising approach to address this problem and protect our farmers from unscrupulous sales of mislabeled or unacceptable impurity of hybrid seeds.

Keywords: hybrid rice, microsatellite, polyacrylamide gel electrophoresis, DNA fingerprinting

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DNA MARKER-AIDED BREEDING FOR RESISTANCE TO RICE BACTERIAL BLIGHT AND TUNGRO DISEASES

Ma. Gina M. Babb*, Ma. Johna C. Duque, Ivy M. Modina, Rustom C. Braceros, Jose Yapchiongco, Jr., Tolentino Rivera and Thelma F. Padolina

Plant Breeding and Biotechnology Division
Philippine Rice Research Institute
Maligaya, Science City of Muñoz, Nueva Ecija 3119
Telefax: +63 44 456 (0277, 0285, 0258) local 260).
*Email: ginababb@philrice.gov.ph; maramarababb@yahoo.com

Bacterial blight (BB) and Tungro diseases have been reported as serious constraints in rice production in irrigated and rainfed lowland environments in the Philippines. Popular varieties IR64, BPIRi10 and PSBRc14 have been highly acceptable to farmers for their good grains, good eating quality and high yielding ability, however, they are susceptible to these diseases. Improvement on bacterial blight and tungro resistance on these varieties was done by exploiting DNA marker-aided selection technique. PhilRice-bred elite lines with pyramided genes *Xa21, xa5,* and *Xa25* for BB resistance in IR64, PSBRc14 and BPI-Ri-10 genetic background have been generated using DNA markers and were used as sources of BB resistance together with IRRI-BB pyramided near isogenic lines IRBB61/62. For tungro, Matatag lines and ARC11554 were used as sources of resistance. Crosses were made and DNA markers were used to select plants with BB resistance genes *Xa7* and *Xa21,* and phenotyping through inoculation using BB races 3 and 6. On the other hand, rice tungro spherical virus resistance was noted by positive amplification with RM 8213. Six out of the 69 populations were positive while twenty-three plants were heterozygous to the *Xa21* gene. Moreover, twenty-five of the populations had the presence of *Xa7* gene. These plants are putatively resistant to all known races of *Xanthomonas oryzae pv. oryzae* in the Philippines. Among all of these plants, fourteen were consistent in their resistance or moderate resistance reaction to BB races 3 and 6 and had the presence of *Xa21* and *Xa7* genes. For tungro, seventeen plants were positive and twenty-one were heterozygous to the RTSV resistance gene. All in all, ten plants were selected that contain the two BB resistance genes and RTSV resistance gene. The elite lines that will be produced in the long run will become bacterial blight-and tungro- resistant varieties. Plants with two or more resistance genes have higher level of resistance to BB and tungro diseases than would be expected from the sum of the parental varieties.

Keywords: bacterial blight, DNA-MAS, rice, tungro

BSD-10

DNA MARKER-AIDED BREEDING FOR RESISTANCE TO RICE BACTERIAL BLIGHT AND TUNGRO DISEASES

Ma. Gina M. Babb*, Ma. Johna C. Duque, Ivy M. Modina, Rustom C. Braceros, Jose Yapchiongco, Jr., Tolentino Rivera and Thelma F. Padolina

Plant Breeding and Biotechnology Division
Philippine Rice Research Institute
Maligaya, Science City of Muñoz, Nueva Ecija 3119
Telefax: +63 44 456 (0277, 0285, 0258) local 260).
*Email: ginababb@philrice.gov.ph; maramarababb@yahoo.com

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Keywords: bacterial blight, DNA-MAS, rice, tungro

BSD-12

PROPIONIC ACID AND METHYLAMINE AS DETOXICANTS OF AFLATOXIN IN COPRA MEAL

Ida F. Dalmacio and Irene A. Papa*

National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños, College, Laguna 4031
Tel.: (049) 536-2724; E-mail: iwapapa@yahoo.com

Aflatoxins are one of the most carcinogenic substances known. Aflatoxin contamination is a major problem in agriculture because aflatoxin-producing *Aspergillus parasiticus* and *Aspergillus flavus* are common and widespread in nature and as such can easily colonize and contaminate agricultural crops before harvest and during storage.

The efficacy of propionic acid and methylamine to detoxify aflatoxin in copra meal was evaluated. Aflatoxin-contaminated copra meal samples were treated with varying concentrations of propionic acid and methylamine and then assayed for aflatoxin at several time intervals to determine aflatoxin content reduction.

Propionic acid at 2% (v/w) caused the highest percent reduction of aflatoxin B1 at 70.87% when meal was treated for 7 days at room temperature. When coupled with heat at 100°C for 90 minutes, 2% (v/w) still caused the highest reduction of aflatoxin B1 at 79.73%. On the other hand, highest percent reduction of 99.37% was attained when methylamine at 2% (v/w) was used. This reduction was not significantly different when a 1.5% methylamine (98.22% reduction) was utilized in the treatment of aflatoxin for 90 minutes at 100°C.

A bench scale study of aflatoxin detoxication in copra meal confirmed the efficacy of methylamine (1.5% v/w) in reducing aflatoxin contamination in copra meal.

Keywords: aflatoxins, *Aspergillus parasiticus, Aspergillus flavus,*, methylamineamine, propionic acid

BSD-13

CYTOGENETICS AND MORPHOLOGICAL ANALYSES OF NINE ACCESSIONS OF SUGARCANE (SACCHARUM OFFICINARUM L.) FROM THE PHILIPPINES

Merlyn S. Mendioro*, Ma. Genaleen Q. Diaz, Donalyn S. Barcial, Flora Joy J. Boy, Lisette Dorothy M. Ocampo, and Luisa N. Villamael

Genetics and Molecular Biology Divison
Institute of Biological Sciences, College of Arts and Sciences
University of the Philippines Los Baños, Laguna,
Tel: 049-536-3368; Fax: 536-2517; E-mail: msmendioro@yahoo.com

Nine Philippine accessions of sugarcane (Saccharum officinarum L.) were morphologically and cytogenetically analyzed using acetocarmine squash technique. Fifty cells each of diakinesis, metaphase I, anaphase I and telophase I were observed and chromosome number determined at diakinesis. Cytological abnormalities were also determined. Morphological analysis was done by the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding. The different accessions showed a range of chromosome number. Mean chromosome number of VMC accessions ranged from 86-96, while Phil. accessions showed 98-108. VMC 68-774 showed the highest mean of 96 while Phil 80-5874 showed 108. Although cytogenetic abnormalities like laggards and non-congression were noted at metaphase I, anaphase I and telophase I, these occurred at low frequencies. Normal metaphase I ranged from 60-100% for VMC accessions while 90-100 % for Phil. accessions. Normal anaphase I ranged from 74-100 for VMC while 76-92 for Phil. accessions. Normal telophase I for VMC and Phil. accessions ranged from 80-100 and 78-100%, respectively. Lagging and non congression of chromosomes were noted but the chromosomes managed to catch-up with the others towards the opposite poles. This explains why a high frequency of normal telophase I was noted. For morphological analysis, VMC68-774 had the highest plant height of 453.5 cm. and the longest stalk length of 337 cm. Phil 64-21 gave the highest plant height of 466.6 cm for Phil. accessions and the longest stalk length of 352.3 cm. VMC 81-21 gave the highest mid internode diameter of 2.89 cm while 3.03 cm was noted for Phil 80-5874. VMC 81-21 had the highest average brix reading of 16.72 while 20.88 for Phil. 89-1233. Accessions with long, wide stalk diameter and high brix reading have potential use for our sugarcane industry. These include VMC 68-774, VMC 68-438, Phil 64-21, and Phil 89-1233.

Keywords: sugarcane, cytogenetics, laggards, internode length, noncongression

BSD-14

PLANTLET REGENERATION FROM CELL SUSPENSION CULTURES OF BANANA CV. 'SABA' VIA SOMATIC EMBRYOGENESIS

Evalour T. Aspuria and Russell John C. de Juras

Crop Science Cluster, College of Agriculture
University of the Philippines Los Baños, College, Laguna 4031, Philippines
Tel.: (049) 536-2807; *E-mail: etaspuria@uplb.edu.ph or etaspuria@yahoo.com;
E-mail: rjcdejuras@uplb.edu.ph

Bananas and plantains (Musa sp.) are among the important fruit crops in the world. Its biological and genetic complexities limit improvement of these species through conventional breeding methods, hence, genetic engineering can be used which relied on efficient regeneration system particularly through somatic embryogenesis. This research was undertaken to generate plantlets from cell suspension cultures via somatic embryogenesis in 'Saba' cultivar.

Sterile shoot tip cultures were obtained from air-dried 'Saba' suckers that were double sterilized with 5% calcium hypochlorite solution for 20 minutes. Highest number of meristematic buds (scalps) proliferated in basal region of shoot tip cultures in modified MS + 1 μM IAA + 100 μM BA in less than 3 weeks of incubation. Treatment with TDZ (1 μM) produced higher number of scalps than with BA (22.2 μM), suggesting that TDZ was a better cytokinin over BA in inducing formation and maintenance of good quality scalps. Yellowish and glossy meristematic globules formed from enlarged scalps in ½ MS macro and Fe-EDTA with 5 μM 2,4-D + 1 μM zeatin. Refreshing the culture medium every 2 days for the first 2 weeks of culture effectively reduced browning of scalps. Embryogenic cells released from meristematic globules were induced to undergo embryogenesis in liquid MS + 9.1 μM zeatin. Primary embryo development was already evident after 2 weeks of incubation, which when transferred onto semi-solid MS + 9.1 μM zeatin formed secondary embryos. This indicates that a change in physical property of the medium further enhanced growth of embryos. Secondary embryos that germinated in the latter medium which were transferred and subcultured 4 times onto MS + 1 μM IAA + 22.2 μM BA produced an average of 7.6 shoots.

To our knowledge, this is the first report on plantlet regeneration from cell suspension cultures on local banana cultivar.

Abbreviations: MS Murashige and Skoog, BA Benzyladenine, Fe-EDTA Iron-ethylenediaminetetra-acetic acid, IAA Indole acetic acid, TDZ Thidiazuron

Keywords: *in vitro*, meristematic cell suspension cultures, meristematic globules, somatic embryogenesis, 'Saba',

BSD-15

FIELD SCREENING AND FRUIT EVALUATION OF BC_3 AND BC_2 SIB-CROSSED PAPAYA PLANTS INTROGRESSED WITH PRSV- P RESISTANCE

Simeona V. Siar[1*], Gil A. Beligan[1], Andres Godwin C. Sajise[1], Antonio C. Laurena[1], Roderick A. Drew[2] and Christopher M.O'Brien[2]

[1] Fruit and Ornamental Crops Section
Crop Science Cluster-Institute of Plant Breeding, College of Agriculture
University of the Philippines Los Baños, College 4031, Laguna
[2]School of Biomedical and Molecular Sciences
Griffith University, Nathan Campus, Brisbane 4111,
Queensland, Australia
Tel. 049-536-8750; Fax 049-536-3438; *Email: moninasiar@yahoo.com

Field performance of BC_1 and BC_2 sib-cross plants against papaya ringspot virus (PRSV-P) and its fruit quality were evaluated. BC_1 plants are product of introgressing the PRSV-P trait from papaya wild type *Vasconcellea quercifolia* to *Carica papaya*. BC_2 sib-crosses were developed by sib-crossing selected female and male BC_2 plants. Selection was based on ELISA test and symptom development in the field.

Six hundred thirty-four backcross plants were inoculated three times at two-week interval in screenhouse. Three hundred twenty-five backcross plants showed typical symptoms ranging from distortion of young leaves, mosaic, chlorosis to shoe-stringed on older leaves. Plants that remained symptom free together with susceptible check, Davao Solo (DS), were then transplanted in the field and were assessed for resistance/susceptibility to Philippine strain of PRSV-P. One hundred-fifteen backcross plants were planted in Mainit, Bay, Laguna. Results showed variation of symptom development in backcross lines from DS. DS produced severe symptoms after 1-2 months in the field while backcross plants remained symptom free for about 7-8 months. Difference between backcross papaya and DS was also evident in the ability of trees to bear good fruits. DS produced few small and unmarketable fruits. Backcross plants in contrast to DS had the ability to recover from early infection based on visual inspection and ELISA test.

Fruit qualities of backcross plants and DS were evaluated. Fruit weight of backcross plants ranged from 834.53-754.92 grams in contrast with DS's 202.67 grams. Fruits have firm yellow orange flesh, with mild papaya aroma. TSS (°B) values of BC_3, BC_2 sib-cross lines and DS were 10.0, 12.2, and 9.3 respectively which corresponds to sweet taste for backcross lines and not so sweet for DS.

The promising result of backcross plants produced by conventional breeding could provide a sustainable approach in restoration of Philippine papaya industry previously devastated by PRSV-P.

Keywords: Papaya ringspot virus (PRSV-P), backcrossing, ELISA, resistance, susceptibility

BSD-16

PRELIMINARY SCREENING OF MALUNGGAY (*Moringa oleifera*) EXTRACT AGAINST *Streptococcus mutans* B-10231, DENTAL PLAQUE CAUSING ORGANISM

Teofila. O. Zulaybar[1], Edren Magdangan[2] , Liezel Gallardo[2] and Lourdes Gatapia[2]

[1]National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños, College, Laguna
[2]Pedro Guevarra Memorial National High School
Telefax: (049) 536-2724; Email: teofilazulaybar@yahoo.com

Malunggay (*Moringa oleifera Lam*) is very famous for its rich nutritive value and remedies for different ailments. The flowers, leaves and roots are used in folk remedies for tumors. Root juice is applied externally as rubefacient or counter irritant. Leaves are applied to sores, rubbed on the temples for headaches and said to have purgative properties (Hartwell, 1971). There are other good uses of malunggay and several researchers reported the production of active compounds from malunggay prevented the growth of pathogenic organisms.

This study considered the oral diseases, such as dental caries and periodontal disease, as consequences of ecological driven imbalance of oral microorganisms. The control of these organisms like *Streptococcus mutans* is fundamental to the maintenance of oral health and prevention of dental caries.

The leaves and seeds of malunggay were extracted with water and 95% ethanol by blending equal parts of malunggay with water or ethanol (1:1), filtered and assayed by paper disc method against *S. mutans* B-10231 as test organism. Ethanol extract of malunggay seeds produced the highest zone of inhibition (zoi) (12.28 mm.dia.) Against *S. mutans* B-10231 as test organism. Ethanol extract of malunggay seeds produced the highest zone of inhibition (zoi) (12.28 mm.dia.) against *S. mutans* which is not significantly different than the positive control (commercial mouthwash), 12.3 mm. zoi. However, the ethanol extract of leaves has 11.26 mm zoi is not significantly different than the water extract of seeds (11.34 mm). The lowest zoi was produced by water extract of leaves (10.8mm). Results showed that the ethanol extract of malunggay seeds can be used as mouthwash which is much cheaper and readily available. These findings revealed that active compounds from malunggay is effective against *S. mutans*.

Keywords: Malunggay, *Streptococcus mutans*, zoi, dental plaque, mouthwash

BSD-17

IN VITRO CALLUS FORMATION IN COTYLEDONS OF MALUNGGAY (*Moringa oleifera*)

Mylene C. Nieves[1*] and Evalour T. Aspuria[2]

[1]Research Office, Central Luzon State University
Science City of Muñoz, Nueva Ecija.
Tel.: 044-456-0704; *E-mail: mye.nieves@yahoo.com.ph
[2]Department of Horticulture, University of the Philippines,
Los Baños, Laguna.
Tel.: 049-536-2807; E-mail: etaspuria@uplb.edu.ph

Moringa oleifera, a small to medium-sized tree, contains important secondary products with anti-oxidant properties which can inhibit tumor cell growth and cure certain chronic diseases. *In vitro* propagation is an important tool for mass propagation and enhancement of secondary metabolites. This study aimed to develop *in vitro* protocol for callus and shoot induction from green mature seeds using cotyledon explants.

Sterile cultures were established by treating the seeds with fungicide, double sterilized with 5% CaOCl for 30 minutes, and disinfected with 1% streptomycin solution. After 5 days of incubation in MS basal medium (MS), 89% sterile cultures were obtained.

Callusing was induced using MS + 2,4-D (0-5.0 µM) + BA (0-2.5 µM) with and without 5.0 µM TDZ, however, highest (97%) callus formation was observed in MS + 2.5 µM 2,4-D + 0.5 µM BA + 5.0 µM TDZ. Loose crystalline calli were observed in MS with 2,4-D and BA singly or in combination, whereas compact, nodular and loose types of calli were obtained in the same media formulations with TDZ suggesting that calli from the latter treatments maybe potential material for shoot induction. High callus weights were observed in MS with 1.0 µM 2,4-D + 0.5-1.0 µM BA, 2.5 µM 2,4-D and BA, and 5.0 µM 2,4-D + 0.5 µM BA enriched with 5.0 µM TDZ. Microscopic observation on green calli after 4 weeks of incubation revealed heterogenous and asynchronous cell growth showing actively dividing cells. Xylem differentiation was evident, indicating early growth of bud initials. It is therefore suggested that transfer of calli showing early bud initiation onto shoot induction media containing high concentration of cytokinin or combination with other plant growth regulator maybe necessary for further organogenesis.

Abbreviations: CaOCl Calcium hypochlorite, MS Murashige and Skoog, BA Benzyladenine, 2,4-D - 2,4-dichlorophenoxyacetic acid, TDZ Thidiazuron

Keywords: *Moringa oleifera* Lam., malunggay, *in vitro*, callus induction, dividing cells, xylem differentiation

BSD-18

Garcinia mangostana (MANGOSTEEN) RIND EXTRACT AS A NOVEL RADIOPROTECTOR AGAINST WHOLE-BODY GAMMA IRRADIATION OF *Mus musculus*

Antonio Miguel Dela Paz[1], Bryan Cassidy Tan[1], Jay Dalet[1*] and Florencio-Isagani Medina III[2,3]

[1]Department of Biology, College of Arts and Sciences
University of the Philippines Manila, Manila, Philippines
[2]Philippine Nuclear Research Institute, Quezon City, Philippines
[3]De La Salle Araneta University, Malabon City, Philippines
*E-mail: jtd0622@yahoo.com.ph

The radioprotective potential of various concentrations (0, 50, 500 and 1000 mg/kg b. wt.) of orally-administered powdered Garcinia mangostana rind extract (GMRE) mixed in distilled water was studied in mice whole-body exposed to 5.0 Gy of γ-irradiation. As assayed through the Micronucleus Test, it was observed that all doses of GMRE considered administered 8 days prior to irradiation conferred a radioprotective effect. The frequency of micronucleated polychromatic erythrocytes (MPCEs) was significantly decreased (p=0.00). Mice administered 0 mg/kg GMRE had the highest mean MPCE frequency (8.83 ± 2.730); followed by 50 mg/kg (3.93 ± 1.174), 500 mg/kg (1.97 ± 1.273), and then by 1000 mg/kg (0.39 ± 0.778). The radioprotective action of GMRE increased in a dose-dependent manner up to 1000 mg/kg, where the smallest number of MPCEs formed (0.39 ± 0.778). It showed no significant difference from the negative control (p=1.00). The high concentration of xanthones present in GMRE possibly conferred radioprotection through free-radical scavenging and immunomodulatory mechanisms. This study demonstrates that orally-administered GMRE, which protected mice against γ radiation-induced cell damage at a maximally effective dose of 1000 mg/kg, could be a novel radioprotector, with advantages of low cost, non-toxicity and high efficiency.

Keywords: *Garcinia mangostana*, Mangosteen, Mice, Micronucleus Test, Radioprotection

BSD-19

ISOZYME POLYMORPHISM IN SOME PHILIPPINE NATIVE ORCHIDS: *Dendrobium anosmum Lindl., Dendrobium sanderae Rolfe, Cymbidium finlaysonianum Lindl., and Cymbidium aliciae Quis*

John Eduard S Acebedo, Rosalina N. Tandang, and Ma. Carmina C. Manuel*

Genetics and Molecular Biology Division, Institute of Biological Sciences
University of the Philippines Los Baños, College, Laguna 4031, Philippines
Tel.: (049) 536-3368; *E-mail: mcdcmanuel@yahoo.com

Isozyme polymorphism in *Dendrobium anosmum Lindl.*, *D. sanderae Rolfe*, *Cymbidium finlaysonianum Lindl.*, and *C. aliciae Quis* was analyzed based on four enzyme systems: acid phosphatase (ACPH), esterase (EST), malate dehydrogenase (MDH), and malic enzyme (ME) using starch gel electrophoresis. Isozyme banding patterns showed a total of seven presumptive loci: three isoloci for EST (*EST-1*, *EST-2*, and *EST-3*), two for ACPH (*ACPH-1* and *ACPH-2*), one each for MDH (*MDH-1*) and ME (*ME-1*). The degree of genetic variability in the four species was determined by estimating the proportion of polymorphism (P), average allele per locus (A), average heterozygosity (H), genetic identity (I_N), genotypic similarity (I_H), and genetic distance (D). The P values of *D. sanderae* (20.00%) and *C. aliciae* (50.00%) were quite low compared to *D. anosmum* (83.33%) and *C. finlaysonianum* (71.43%). It was also noted that the H values of the four orchid species were below 50%. The low P and H values suggest that there is low intraspecific variation in each of the four species. Based on the enzyme loci surveyed, *D. anosmum* and *C. finlaysonianum* had approximately two alleles per locus (average 1.774) while *D. sanderae* and *C. aliciae* had approximately one allele per locus (average 1.35). The computed estimates for genetic variation showed that the least genetic identity (I_N = 0.565), lowest genotypic similarity (I_H = 0.466), and the greatest genetic distance (D = 0.571) occurred between *D. sanderae* and *C. finlaysonianum*. This could be attributed to the inherent genetic differences between the two genera. One notable difference was the presence of *EST-3* locus in the two *Cymbidium* species and absence in the two *Dendrobium* species. However, *D. anosmum* and *C. finlaysonianum* exhibited the greatest genetic identity (I_N = 0.793), highest genotypic similarity (I_H = 0.641) and the lowest genetic distance (D = 0.232) even though they belong to different genera. It is possible that the variation between these two genera was not represented well based on the four enzyme systems used. The use of other enzyme systems is therefore recommended.

Keywords: *Dendrobium* sp., *Cymbidium* sp., orchids, isozyme polymorphism, starch gel electrophoresis, genetic variation

BSD-20

TOWARDS THE CATALOGUING OF THE PHENYLALANINE AMMONIA LYASE (*PAL*) AND CAFFEATE-*O*-METHYLTRANSFERASE (*COMT*) GENES FROM THE RHIZOMES OF *ZINGIBER OFFICINALE* ROSC. AND *ALPINIA GALANGA* (L.) SW.

Kevin Kiel V. Apeles, Greg Matthew E. Teo, Neil H. Tan Gana*, and Vivian P. Panes*

Department of Biology
Science Education Complex, Ateneo de Manila University
Katipunan Avenue, Loyola Heights, Quezon City 1108, Philippines
Tel.: +632 426-6001 loc. 5613; TeleFax: +632 426-1034;
*E-mail: ntangana@ateneo.edu, vpanes@ateneo.edu

This study aims to provide the groundwork in constructing a gene catalogue that encodes for the two primary enzymes of the phenylpropanoid-hydroxycinnamate pathway, *phenylalanine-ammonia lyase (PAL)* and *caffeate-O-methyltransferase (COMT)*, which are responsible for the subsequent synthesis of the medicinal secondary metabolites as an example is gingerol, of *Zingiber officinale* and *Alpinia galanga*. The designed gene-specific primers for PAL (forward primer, 5'-TTCAAGATCGCCGGCATCGA-3; and reverse primer-5'-GTTCCACTCCTTGAGGCACTCGAG-3'), and COMT (forward primer, 5'-CAACGGGCCTACTGTCATTCG-3'; and reverse primer, 5'-TTCAAGATCGCCGGCATCGA-3'), enabled the amplification of polymerase chain reaction (PCR) products from genomic DNA and total RNA of both plants. Results revealed that genomic DNA PCR amplicons are expressed as single copies in both plant genomes with sizes 1,700 bp for COMT and 1,300 bp for PAL. Reverse transcription polymerase chain reaction (RT-PCR) of *Zingiber officinale* total RNA yielded four putative cDNA isoforms for PAL with sizes 1,400 kb, 700 kb, 550 kb and 300 kb. With this is inferred that at the replication level, *Comt* and *Pal* genes in both plants are expressed as single copies in both genomes indicating that these enzymes are highly regulated. However, at the transcription level, *Pal* gene in *Z. officinale* is expressed as four putative cDNAs for isoforms of PAL indicating that molecules of the same family perform different functions in the phenylpropanoid-hydroxycinnamate pathway to synthesize medicinal secondary metabolites, and molecules of the same function performing in other pathways to synthesize other medicinal secondary metabolites. This study is the first to report of possible alternative splicing mechanisms in the gene expression of PAL in *Z. officinale* suggesting probable regulatory functions in the alternative phenylpropanoid-hydroxycinnamate pathway.

Keywords: phenylpropanoid-hydroxycinnamate pathway, phenylalanine-ammonia lyase (PAL) and caffeate-O-methyltransferase (COMT)

BSD-21

SEQUENCE ANALYSIS OF *VIBRIO HARVEYI TOXR* GENE FOR INSIGHTS ON ITS POSSIBLE ROLE IN PATHOGENICITY

Prima Fe R. Franco[1*] and Cynthia T. Hedreyda[2]

[1]Department of Biology, College of Arts and Sciences
Mariano Marcos State University, Batac, 2906, Ilocos Norte
*Email: primarfranco@gmail.com
[2]National Institute of Molecular Biology and Biotechnology
College of Science, University of the Philippines, Diliman, Quezon City

The gene *toxR*, an ancestral gene of Family Vibrionaceae, codes for the protein ToxR, a transmembrane transcription regulator of several virulence factors. This study explored the possibility of distinguishing pathogenic from non-pathogenic *Vibrio harveyi* strains at the molecular level using *toxR* as gene marker.

Complete *toxR* gene sequence of type strain *V. harveyi* NBRC 15634 was obtained after amplifying 5. and 3. regions flanking a 576-bp *V. harveyi* gene fragment previously sequenced.. A 750-bp terminal 5. *toxR* gene sequence was from an amplified fragment using a forward primer (*VhtoxRpv)* based on known 5. *toxR* sequences in *V. parahaemolyticus* and *V. vulnificus* and a reverse primer (*VctoxR2R*) targeting an internal region of the 576-bp fragment.

The 900-bp 3. terminal *toxR* region was amplified using a primer pair *toxRS1* and *toxRS 2* based on *V. parahaemolyticus* and *V. vulnificus toxR* and *toxS*. Sequence analysis and alignment of the complete 882 bp *toxR* revealed that *V. harveyi* shares 87% similarity with *toxR* of *V. parahaemolyticus*, 84% with *V. fluvialis*, 83% with *V. vulnificus*, and 74% with *V. campbelli*, indicating wider divergence in *toxR* compared to 16S rRNA gene among *Vibrios*. Nucleotide sequence comparison of the *toxR* from the pathogenic versus the non pathogenic strains revealed significant nucleotide sequence variation from the type strains *V. harveyi*, including a 19 bp deletion in one non-pathogenic isolate and mutations that resulted in stop codons in the other three *toxR*. Significant protein sequence variation was observed between the pathogenic and non-pathogenic *V. harveyi*, resulting in mutations that affected protein structure associated with the periplasm. While hemolysin gene and protein sequences were comparable among pathogenic and non-pathogenic *V. harveyi* strains used in this study, variations in *toxR* might have affected the function of the ToxR gene product, implicating *toxR* gene mutations to loss of pathogenicity.

Keywords: periplasm, *toxR*, Vibrionaceae, *Vibrio harvey* PCR, hemolysin, mutations, p sequence analysisathogenicity *V. campbelli*

BSD-22

HRP B GENE SEQUENCES REFLECT THE ORIGIN AND HOST RANGE OF *RALSTONIA SOLANACEARUM*

Joselito E. Villa[1*], Kenichi Tsuchiya[2], Mitsuo Horita[3], Neilyn O. Villa[4] and Mitsuro Hyakumachi[5]

[1]Crop Protection Division, Philippine Rice Research Institute
Science City of Munoz, Nueva Ecija, Philippines
*E-mail: joeyvilla@yahoo.com
[2]Laboratory of Plant Pathology,
Department of Applied Genetics and Pest Management
Kyushu University,6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[3]National Agricultural Research Center for Hokkaido Region
Hitsujigaoka 1, Toyohira, Sapporo, Hokkaido 062-0045, Japan
[4]Genetics and Molecular Biology Division, Institute of Biological Sciences
University of the Philippines Los Baños, College, Laguna, Philippines
Tel.: 09186283949 or 049 536 3368; *E-mail: neilyn24@yahoo.com
[5]Laboratory of Plant Disease Science, Gifu University,
1-1Yanagido, Gifu, Japan

Ralstonia solanacearum is an important plant pathogen, causing bacterial wilt of vegetables and banana plants. Knowledge of their genetic diversity is very important in order to understand their origin, host range, and for implementation of proper control measures. The h*rpB* gene is vital for the pathogenicity of *R. solanacearum*. *HrpB* gene encodes the regulatory protein that controls the expression of the Type III Secretion Apparatus (TTSS) together with several effector proteins. In this study, we have analyzed the *hrp B* sequences from different strains of *Ralstonia solanacearum* as they pertain to the origin and host range of the strains. *HrpB* genes of different isolates of *R. solanacearum* in the Philippines were amplified by PCR using specific primers for *hrpB* gene. The amplified *hrpB* genes were purified, sequenced and analyzed. Additional *hrpB* sequences of *R. solanacearum* from other countries, blood disease bacterium (BDB), and *Pseudomonas syzygii* from Indonesia that were previously reported were included in the analysis. Based on the results, the strains were divided into 4 clusters or phylotypes. Race 1 strains, wide host range, were found in phylotype 1. Race 2 strains, typical potato strains and banana strains, were in phylotype 2. Asian biovar N2 from potato, the isolates from clove, BDB and *Pseudomonas syzygii* were all grouped in phylotype 4. Phylotype 3 contained strains exclusively isolated from Africa. Phylotypes 1 (Asian) and 3 (African) are most closely related than to other phylotypes. The results showed the very diverse characteristic of *R. solanacearum* strains found in Asia as they were present in three out of the four phylotypes. Biovar N2 strains were very different from the other strains infecting potato in the Philippines. The origin and host range of this relatively new strain entails more research to understand their importance.

Keywords: HrpB gene, biovar classification, Ralstonia solanacearum

BSD-23

NITROGEN-FIXING BACTERIA ASSOCIATED WITH THE ROOTS OF PHILIPPINE SHALLOT 'SIBUYAS TAGALOG' (*Allium ascalonicum*)

Maria Carissa D. Martir*[1], Vivian A. Panes[1], Wilfredo L. Barraquio[2†]

[1]Department of Biology
School of Science & Engineering, Ateneo de Manila University
Katipunan Avenue, Loyola Heights Quezon City, Philippines 1108
Tel: (+632)4266001 loc 5612; Telefax: (+632)4261034;
Mobile: (+63)9189006156; *E-mail: mmartir@ateneo.edu
[2]Institute of Biology, College of Sciences
University of the Philippines Diliman, Quezon City, Philippines1101
[†]Deceased

This paper reports nitrogen-fixing bacteria associated with a top exportable vegetable, Philippine shallot (Allium ascalonicum) or Sibuyas Tagalog, with the hope of using them as biofertilizer amendment to organic fertilizers for onion farming in the future.

The nitrogen-fixing bacteria associated with roots of Sibuyas Tagalog were enumerated and isolated using the MPN technique combined with acetylene reduction assay (ARA). The MPN population of nitrogen-fixing bacteria was found to be 5.22×10^4 and 1.80×10^5 per gram root dry weight, in malate and in glucose semi-solid media respectively. Isolates from ARA-positive MPN cultures were found to have specific nitrogenase activity that ranged from 334-450 nmol C_2H_4 mg protein^{-1} hr^{-1}. Strain RdG1 was characterized to be a Gram negative short rod, motile, resistant to several antibiotics, non-spore forming, non-fermentative, non-nitrate reducing, and able to utilize a wide variety of organic compounds similar to the root exudates of plants. In addition, RdG1 fixes nitrogen under microaerobic conditions the same oxygen levels as would be found in roots and rhizosphere of plants. It is interesting to note that no Azospirillum spp. nor diazotrophic enterobacteria have been isolated from Sibuyas Tagalog roots in this study.

API, Biolog, and manual tallying of phenotypic data from literature showed that RdG1 has similarities to Burkholderia. However, percentage similarity of RdG1 to B. cepacia based on phenotypic characteristics is relatively low. There is the possibility that RdG1 may be a novel strain or species. Unlike B. cepacia, RdG1 is not pathogenic to onion.

Keywords: Nitrogen-fixing Burkholderia, Allium ascalonicum auct, Allium cepa var. grp. aggregatum, diazotroph, Sibuya Tagalog, **biofertilizer**

BSD-24

PHENOTYPIC PROFILE OF HETEROTROPHIC BACTERIA IN *Penaeus monodon* POND SEDIMENT, REARING WATER AND WATER SOURCE IN ONE REARING CYCLE

Angeli A. Valera and Buenaflor D. Jimenez

Department of Biological Sciences
Mindanao State University-Iligan Institute of Technology, Iligan City
Tel.: (063)221-4050 loc 137; E-mail: angeli_valera@yahoo.com

Profile of heterotrophic bacteria in *Penaeus monodon* pond sediment, rearing water and water source of BFAR-NFRDI ponds at Pacita, Lala, Lanao del Norte was determined within one rearing cycle. Sampling was done weekly in the four designated sites: site A (water source), site B and C (ponds with *P. monodon*) and site D (pond without *P. monodon*). Composite samples of water and sediment were collected and cultured in nutrient agar medium by serial dilution and streak plate method. Isolates were then purified and subjected to biochemical tests.

A total of 185 isolates were obtained. Gram-negative bacteria were higher in sediments (58-63%) than in the water samples (54-60%). Vibrionaceae had the most number of isolates in water samples (7 to 13 isolates). Micrococcaceae had the most number of representative isolates and highest density for several weeks in the sediments of the reared and undreared ponds, respectively. Decrease in both diversity and evenness in the rearing water were observed after introduction of feeds. In this period, Vibrionaceae and Bacillaceae dominated in the reared ponds. Diversity and evenness decreased on the fifth week in sediment samples. Ratio of Vibrionaceae against other bacterial groups was generally higher in the rearing water than the pond sediments. The abundance of bacterial groups is influenced by presence of other biotic components, prawn rearing practices, and water quality within the ponds.

Keywords: heterotrophic bacteria, *Penaeus monodon*

BSD-25

A RAPID IN VITRO CALORIMETRIC METHOD OF DETERMINING BACTERICIDAL POTENCY OF ANTIMICROBIALS

Ronald R. Matias*, Leilani Baltazar, Wilfredo Verar Jr., Mark Ritumalta, Frederick dela Cruz, Madeliene Fabros, Leila Florento, Mario Panaligan and Alexander Tuazon

Biological Sciences Department
Medical Affairs Division, United Laboratories Inc.
Tel.: (632) 631-8501 local 7423; *E-mail: rrmatias@unilab.com.ph

The increase of antibiotic-resistant microorganisms possibly leading to severe clinical infections necessitates the development and application of rapid and accurate antimicrobial susceptibility assays. These new tests can be used to screen natural products for potential antimicrobial properties. These assays are also used to determine bactericidal potency, a very important parameter for the proper evaluation of generic antibiotics. Colorimetric based assays are rapid alternative methods for antimicrobial susceptibility testing. This involves the reduction of a tetrazolium salt by metabolically active cells to a colored water-soluble formazan derivative and quantified using a spectrophotometer. In this study the bactericidal potency of a generic antibiotic was compared to a branded commercial product. The bactericidal potency was characterized using the American Type Culture collection (ATCC) reference strains as recommended by the CLSI. Furthermore, bacteria isolated from different clinical cases were also used. The bactericidal pharmacodynamics of the various antibiotics against the different target microorganisms was analyzed by generating a concentration-killing-curve (CKC). Half-maximal effective concentration (EC_{50}) of the different antimicrobial agents was obtained from an experimentally derived dose-response curve. Potency of the different antibiotics was statistically calculated using Parallel Line Assay. Different bacterial strains show different CKC profiles but no difference in potency was observed between the generic and branded commercial antibiotic. Difference in sensitivities between ATCC strains and clinical isolates of the same genera were observed. *In vitro*-based antimicrobial susceptibility assay promises to be a useful and rapid method for evaluating potency of generic antibiotics. Furthermore, it is recommended that bacterial isolates from clinical cases other than ATCC reference strains be used to evaluate antimicrobials.

Keywords: Antimicrobial susceptibility test, Bactericidal Potency, concentration-killing-curve, Generic antibiotic

BSD-26

VALIDATION TRIALS FOR THE PCR-BASED DETECTION OF *E. coli* O157:H7 IN FOODS USING THE LOCALLY-DEVELOPED DNA AMPLIFICATION SYSTEM (DAS) KIT

Maria Teresa M. Perez*, Susana M. Mercado, Marilou R. Calapardo and Margarita A. Mercado

National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Banos, College, Laguna 4031
Telefax: (049) 536 2721; *Email: mtmperez14@yahoo.com.ph

The study aimed to validate the applicability of the BIOTECH UPLB-DOST DAS kit in detecting enterohemorrhagic *E. coli* O157:H7 in fresh produce, meats and dairy products. Uninoculated and artificially-contaminated hamburger patties, white cheese and liquid milk samples were enriched for 24 h at 37°C in modified Tryptic soy broth (mTSB) supplemented with 0.02 mg/ml novobiocin. Vegetable samples were washed in Butterfield's Phosphate buffer and the washings enriched for 20 h in Buffered peptone water + novobiocin. The presence of the target analyte was determined by plating on Tellurite-Sorbitol MacConkey agar and by PCR using the *E. coli* O157:H7 DAS kit. IMViC tests were conducted on presumptive EHEC O157:H7 isolates obtained from white cheese and uninoculated hamburger patties samples. In the analysis of vegetable samples, the DAS result indicated all uninoculated samples as negative and all EHEC O157:H7-seeded replicates as positive. The plating method scored the uninoculated mixed lettuce as presumptive positive and one replicate of seeded carrots and two replicates of seeded salad tomatoes as negative. For the fifteen cheese samples, the percent agreement between the culture method and the DAS kit was 86.67%. Two uninoculated cheese samples yielded the target amplicon however, in the cultural method, none or very few presumptive colonies were detected in these two samples. A total number of 45 colonies from cheese samples subjected to IMViC identified only one, from a seeded sample, as *E. coli*. For the fifteen milk samples, 100% percent agreement was obtained in the parallel testing of the two methods. In the analysis of hamburger patties, all samples tested positive with the *E. coli* O157:H7 DAS kit. Verification of natural contamination of the hamburger patties samples was done by employing both methods prior to enrichment (T_0), after 6 h (T_6) and 24 h (T_{24}) in mTSB + novobiocin at 37°C. A total of 50 presumptive isolates from T_{24} did not show typical *E. coli* reactions with IMViC tests and all 21 isolates tested by PCR were negative for the target amplicon. Because of the difficulty in isolating EHEC O157:H7 from meat microflora for confirmation by cultural method, it was not established whether this batch of hamburger patties samples was indeed contaminated by EHEC O157:H7. Improvement in isolation procedures and analyses with higher sample size are underway.

Keywords: *E. coli* O157:H7, polymerase chain reaction, pathogen detection, detection kit, food safety

BSD-27

COMBINATION OF POLYMYXIN B AND BACTERIOPHAGE F116: AN EFFECTIVE TREATMENT FOR *Pseudomonas aeruginosa* BIOFILM

Erwine S. Dela Paz and Merab A. Chan*

Department of Biology, Ateneo de Manila University
Katipunan Avenue, Loyola Heights, Quezon City 1108, Philippines
Tel.: 426-6001 loc. 5613; E-mail: erwinedelapaz@yahoo.com,
mchan@ateneo.edu

The effects of combined Polymyxin B (PB) and bacteriophage F116 in culture on *Pseudomonas aeruginosa* biofilm was investigated. The study also explored the effectivity of the augmentation on the different stages of biofilm development. *P. aeruginosa* biofilms were grown and the different stages of development were treated with PB, bacteriophage F116, and a combination of PB and phage F116. Cell viability was assessed through 2,3-bis 2-methyloxy-4-nitro-5-sulfophenyl-2 H-tetrazolium-5-carboxanilide) (XTT) assay. Furthermore, the total amount of biofilm (bacterial cells and exopolysaccharide substances EPS matrix) was determined through crystal violet staining. The effectivity of the treatments in degrading the EPS matrix was investigated using the scanning electron microscope (SEM); and deoxyribonucleic acid (DNA) analysis was conducted to assess if there was lysogeny of the biofilm cells that survived the combined treatment. Lysogeny indicates an integration of bacteriophage F116 DNA to the bacterial genome. Twoway analysis of variance was done to confirm if the treatments and biofilm development significantly affected the survival of the biofilm. Results revealed that the combined PB and bacteriophage F116 treatment reduced the mature biofilm's cell viability and total amount by 72% and 75%, respectively compared to PB treatment alone that resulted only in an 11% and 4% reduction in similar assays. SEM investigation confirmed the decrease in the apparent thickness of the EPS matrix with the combined treatments. Lysogeny was also observed in the bacterial cells, suggesting that the bacteriophage treatment alone could be ineffective without PB. Statistical analysis showed that both treatments and stages of biofilm development significantly affect the survival of the biofilm cells.

Keywords: *Pseudomonas aeruginosa* biofilm, Bacteriophage F116, Polymyxin B

BSD-28

EVALUATION OF PRODUCTION SAFETY OF A PORK SAUSAGE VARIANT ("HAMONADO LONGANISA") WITH RESPECT TO *ESCHERICHIA COLI* SURVIVAL THROUGH THE USE OF SELECTED PREDICTIVE MODELS

Martha Hedwig D. Bayer[1]* and Agnes F. Zamora[2]

[1]Institute of Biological Sciences
Tel.: (049) 536-3368; *E-mail: reyabmh@yahoo.com.
[2]Microbiology Division, Institute of Biological Sciences
University of the Philippines Los Baños, 4031 College, Laguna, Philippines

Population kinetics of *Escherichia coli* (BIOTECH 1098[1]) in a commercially available pork sausage variant (hamonado "longanisa") was determined at various temperatures employed during the production process. The hamonado "longanisa" was evaluated for production safety with respect to *E. coli* by using challenge tests and two currently available predictive microbiological models, MiroFit v. 1 (Baranyi Model) and Pathogen Modeling Program 6.1 (PMP 6.1). Results obtained showed that as temperature was increased over time to 35 °C, maximum specific growth rate ($?_{max}$) also increased while a shorter generation or doubling time (t_d) was observed under the conditions tested in this study.

The predictions generated by MicroFit v. 1 (Baranyi Model) and PMP 6. 1 were found to be generally consistent with observed results for the majority of the challenge tests. Bias factors (B_f) and accuracy factors (A_f) were used as comparison indices. Based on the B_f and A_f, the Baranyi Model generated estimates fit more closely to the observed values, particularly for the lag phase and its transition into the log phase of growth for *E. coli*. The obtained results indicate that the Baranyi model may be used to adequately predict end product safety and acceptability in an actual food environment at different temperatures. In general, results of this study indicate that as long as the prescribed time and temperature limits are strictly adhered to and Hazard Analysis Critical Control Point (HACCP) protocols are followed during production, end product safety with respect to *E. coli* for the studied hamonado longanisa variant will be maintained.

Keywords: predictive microbiology, hamonado "longanisa", *Escherichia coli*, MicroFit v.1 (Baranyi Model), Pathogen Modeling Program 6.1

BSD-29

LEAD PHYTOEXTRACTION OF ROBUST PLANT SPECIES PRESENT IN HEAVY METAL CONTAMINATED SITES

Litton S. Halder[1*], Orlex B. Yllano[1,2], Ramon Ulangca Sr.[1*]

[1]Graduate Biology Program and University Research Center
Adventist University of the Philippines
[2]Molecular Biology and Biotechnology Program,
University of the Philippines Los Baños, Laguna, Philippines
Tel.: 049-5411211-28; Fax: 049-5411228;
*Email: lex@aup.edu.ph, obyupd@yahoo.com

Lead contamination of soils is implicated to various environmental problems and toxicity in flora, fauna and humans. Pb levels in plant and soil samples from two military firing ranges were analyzed using Inductively-Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Physicochemical analysis of soil from different distances in site 1 and 2 revealed variable profiles in soil pH, cation exchange capacity (CEC), organic matter (OM), and Pb concentrations. The average Pb concentration in site 2 (3185.17 ppm) was higher compared with site 1 (1173.14 ppm). These Pb concentrations in contaminated sites were two orders of magnitude higher than that of the control site (10 ppm). Correlation analysis indicated that the soil Pb concentrations were positively correlated with soil pH and OM but negatively associated with soil CEC. Likewise, Pb-tolerant plant species belonging to family Poaceae, Leguminosae and Euphorbiacea were identified in both sites with high Pb bioaccumulation in their root and shoot tissues. Pb concentrations in the root and shoot tissues of *Mimosa pudica* (1693.45 ppm), *Dichanthium sericeum* (1486.75), *Centrosema pufescens* (1239.00 ppm), *Eleusine indica* (981.05 ppm), *Panicum antidotale Retz* (851.55 ppm), *Cynodon dactylon* (823.90 ppm), *Cyperus rotundus* (646.60 ppm) *and Ricinus communis* (52.15 ppm) were considerably higher compared to their control counterparts. Correlation analysis showed that total Pb accumulation was positively associated with root and shoot Pb, relative dominance (RD), and relative frequency (RF), and importance value (IV) of the species. *Centrosema pufescens* had the highest bioconcentration factor (BCF) of 0.86. *C. pufescens, M. pudica, Cynodon sp., E. indica* and *P. antidotale R.* were the candidate robust plant species that can hyperaccumulate Pb in their root and shoot tissues. Our results provide compelling evidence on the ability of these robust plant species in extracting Pb from heavy metal contaminated sites.

Keywords: phytoextraction, phytoremediation, robust species, Pb contamination, bioconcentration factor

BSD-30

PHYTOEXTRACTION OF NICKEL BY *BRASSICA JUNCEA* (INDIAN MUSTARD) AND *ZEA MAYS* (CORN) CULTIVATED IN NICKELIFEROUS LATERITE FROM BROOKE'S POINT, PALAWAN, PHILIPPINES

Rene Juna R. Claveria, Maria Katherina C. Patdu and Jewel Racquel S. Unson*

Department of Environmental Science
School of Science and Engineering, Ateneo de Manila University
Loyola Heights, Quezon City 1108
Telefax: (632) 4264321; *E-mail: junson@ateneo.edu

This study tested the growth responses and accumulative capacity of *Brassica juncea* and *Zea mays* to nickel from nickeliferous laterite soil. Addition of compost in one of the soil groups was employed. Concentrations of nickel were measured at 100 ppm (garden soil), 7000 ppm (laterites with compost) and 7600 ppm (laterites).

In evaluating growth responses of plants to nickel, percent shoot and root length difference, growth rate, dry matter production and water content were measured. Visible phytotoxic effects were noted through ocular inspection. Nickel accumulation was quantified through Atomic Absorption Spectrophotometry (AAS). For both species, the bioconcentration factor (BCF) and phytoextraction rate were determined as the measure of the plants' phytoextraction capacity.

In an evaluation of the growth responses of both species to nickel through the measurement of salient parameters, visible and quantifiable results indicated that the addition of the compost was able to alleviate the nickel toxicity in *Brassica juncea* grown in nickeliferous laterites while *Zea mays* plants did not elicit any visible phytotoxic effects.

Nickel toxicity effects observed in *Brassica juncea* were wilting,discoloration (chlorosis) and stunted growth. The effects began to be elicited by the plants during the 3rd week of growth. For *Zea mays*, toxic effects observed were stunted growth. Results reveal that there is an increase in the nickel concentration in plants at higher soil metal concentration. Using the BCF values as the measure of phytoextraction capacity of the plants, *Zea mays* showed medium nickel accumulation while *Brassica juncea* showed slight nickel accumulation.

Keywords: Phytoextraction; nickel uptake; nickeliferous laterites; *Brassica juncea*; *Zea mays*

BSD-31

BIOREMEDIATION POTENTIAL OF FUNGI ISOLATED FROM WASTE SITE SOIL SAMPLES

Erwin P. Elazegui, Madonna B. Piamonte and Eleasar D. Solomon

College of Science
Technological University of the Philippines Manila

Bioremediation is the process by which microorganisms degrade toxic substances to environmentally safe levels in soils and other systems. This study was conducted to determine the bioremediation potential of fungi isolated from the soil sample waste site of Technological University of the Philippines , Manila . A total of four isolates were obtained . One fungal isolate was evaluated for its potential to degrade lead . Pure culture of the organism wsa inoculated into Sabouraud Dextrose Agar (SDA) with lead nitrate of varying concentrations (62.5 ppm , 187.5 ppm and 312.5 ppm) . Mycelial Growth Extension and Mycelial Dry Weight were measured. Fungal species was identified based on its morphology , surface color and texture appearance on SDA. Concentrations of lead in SDA was determined using Atomic Absorption Spectrophotometry. Results showed that *Mucor sp.*, the identified organism can degrade high concentrations of lead.

Keywords : bioremediation , fungi , lead , mycelial growth extension , mycelial dry weight

BSD-32

FUNGAL DIVERSITY AND ETHNICAL VALUES: ROLE IN BIODIVERSITY CONSERVATION of CORON, PALAWAN, PHILIPPINES

Carina R. Capistrano[1], Nelson M. Pampolina[1] and Manuel M. Baldovino[2]

[1]Department of Forest Biological Sciences
College of Forestry and Natural Resources
Telefax: +63 49 536 2773; E-mail: nelmpampolina@yahoo.com
University of the Philippines Los Baños
[2]Museum of Natural History
University of the Philippines Los Baños

Coron as member of Calamian group of islands in Northern Palawan is among the ecotourism hotspot in the Philippines - a home of rich biodiversity resources like fungal flora, which form part of Tagbanua's culture. However, anthropogenic impacts like migration, industrialization, and kaingin farming have caused biodiversity degradation, requiring conservation strategies to restore the island and mountain ecosystems. This research will determine fungal diversity in Coron, assess their ethnical values for Tagbanua, and evaluate conservation strategies that sustain island biodiversity. Fungal diversity and ethnical values from different ecosystems (Boho-forest, kaingin-forest, limestone forest) within Mt Darala and Dungon Islands were investigated in wet and dry periods (2006-2007). A 3-4 km transect was stretched along three ecosystems, where three 10 x 10m quadrants were alternately established at equal interval to characterize fungal morphology for identification and quantify density and diversity indices. Indigenous knowledge on fungal flora was obtained through key informant interview approach.

There were 64 fungal species belonging to 44 genera and five orders (Aphylloporales, Agaricales, Sphaeriales, Tremellales and Thellophorales) grouped into two classes (Basidiomaycetes, Ascomycetes). Fungal density per 100 m^2 was higher in Mt Darala (727 1,074 individuals) than Dungon islands (213 403 individuals). Though even in distribution (e=0.7900), fungal diversity in Mt. Darala was moderate (H'=2.7570) and low in Dungon Island (H'=1.6100) due to human activities. Fungal diversity during wet season was 18.5% higher than collections in dry period, suggesting importance of moisture in their life cycle. Based on respondents, everyone utilized fungi as alternative food while 43% stated their cultural values and 7% uses fungi as biofertilizer. Overall, ethnomycological values indicate possible strategies for sustainable biodiversity conservation by protecting fungal habitat, regulating harvest and consumption of wild fungi, minimize burning during site preparation of upland farms, and delineation of areas for mushroom collection.

Keywords: Fungal diversity, ethnomycology, biodiversity conservation, island and mountain ecosystems, Coron Palawan

BSD-33

MICROBIAL COMMUNITY ASSESSMENT OF MT. MAKILING MUDSPRING, LAGUNA, PHILIPPINES AS REVEALED BY 16S rDNA

Nacita B. Lantican[1], Ma. Genaleen Q. Diaz[1], Jessica F. Simbahan[2], Jose Jason L. Cantera[3] and Asuncion K. Raymundo[1]

[1]Institute of Biological Sciences, College of Arts and Sciences
[2]National Institutes of Molecular Biology and Biotechnology
University of the Philippines Los Baños, Laguna. Philippines
[3]University of California, Riverside CA

During the last decade, molecular biology techniques based on DNA extraction have given microbiologists a powerful tool to explore the microbial communities of extreme environments such as geothermal acidic environments, revealing uncharacterized members belonging to both the bacterial and archaeal domains. Consequently, considering the diversity of potential habitats and the great genetic diversity of microbes, the vast majority, most of which are as yet unknown microbes from solfataric environments, could well be a huge source of novel molecular structures. Mt. Makiling Mudspring is a thermophilic acidophilic environment harboring a wide range of microorganisms waiting to be discovered. This study was the first attempt to study the site using molecular techniques. Efforts were made to extract DNA of a high quality and quantity from SM and SSW samples and to establish the first phylogenetic analysis.

Among the five environmental DNA isolation techniques with several modifications tried, the only method where DNA was successfully isolated was with the BIO 101 Fast DNA Spin Kit for Soil using freshly sampled sediments from the upper layer of solfataric soils with hot spring water (SULSSW5) from Mt Makiling Mudspring. No DNA was isolated from solfataric mud (SM) regardless of the method used.

Phylogenetic analysis using archaeal (23FPL-1391R) and universal (519F - 1392R) primer pairs showed that twelve clones were clustered with *Sulfolobus tokodaii*, and five clones with *S. solfataricus* and *S. islandicus*. Seven clones clustered with clones named MTC-A. One clone, D519-8, had low similarity with uncultured archaeon clone KOZ184; hence, it is believed to be novel. The clones from the bacterial primer pair (11F and 1492R) did not reveal any significant BLAST result.

The cloning and sequencing of environmental 16S rDNA fragments have led to the detection of the *Sulfolobus* group of organisms thriving in the aforementioned environment based on universal and archaeal 16S rRNA analyses. The bacterial clone library did not show any significant group of organisms This is a pioneering work in the use of environmental DNA for the analysis of the microbial community of Mt Makiling, Mudspring and of any environment in the Philippines.

BSD-34

COMMUNITY ASSEMBLAGE OF MICROALGAL EPILITHON IN CAGAYAN DE ORO RIVER

Astrid L. Sinco[1,2*], Ephrime B. Metillo[2] and Adora S. Rondez[1]

[1]Department of Biology, Xavier University
Corrales Ave. Cagayan de Oro City 9000
*E-mail: a.sinco@xu.edu.ph
[2]Department of Biological Sciences
Mindanao State University-Iligan Institute of Technology
A. Bonifacio Ave., Iligan City 9200
*E-mail: ephrime.metillo@g.msuiit.edu.ph

There is a paucity of information on the community assemblage of epilithon in Philippine rivers. We, therefore, conducted a preliminary study of benthic microalgal assemblages in different areas of Cagayan de Oro River which is the major river system of Cagayan de Oro City. We sampled epilithon from riffle and pool sites in upstream, midstream and downstream segments of the river in January 2008. Physical (sampling site elevation) and water conditions (temperature, pH, dissolved oxygen, conductivity, total suspended solids, and velocity) were also determined during sampling. Thirty-five genera comprised the epilithon of Cagayan de Oro River most of which belong to Bacillariophyceae (21 genera) followed by Chlorophyceae (7 genera), Cyanophyceae (6 genera) and Xanthophyceae (1 genus). Most of the genera in Bacillariophyceae were mostly pennate diatoms with *Stauroneis* as the most abundant. *Microspora* was the most abundant among the Chlorophyceae. The Shannon index of diversity was highest in the downstream pool site (H'=2.27) while lowest was in upstream riffle site (H'=1.62). In terms of generic richness, the riffle midstream sites had the highest value (S = 27) and the least was in the pool upstream site (S = 22). Multivariate analysis of data using the Redundancy Analysis (RDA) of CANOCO software version 4.54 showed that only elevation had a significant ($p < 0.01$) influence on the distribution of epilithon genera, with most of the diatoms favoring lower elevations.

Keywords: epilithon, Bacillariophyceae, Chlorophyceae, Cyanophyceae, Chlorophyceae

BSD-35

IDENTIFICATION OF 18S RIBOSOMAL DNA GENOTYPES OF *ACANTHAMOEBA* SPECIES ISOLATED IN THE PHILIPPINES

Windell L. Rivera[1,2*] and Davin Edric V. Adao[2]

[1]Institute of Biology, College of Science
[2]Molecular Protozoology Laboratory, Natural Sciences Research Institute,
University of the Philippines Diliman, Quezon City
Telefax: 920-5471; *E-mail: wlrivera@science.upd.edu.ph

Acanthamoeba is a genus of ubiquitous free-living amoebae which commonly causes the human eye infection *Acanthamoeba* keratitis (AK) as well as the fatal brain infection known as granulomatous amoebic encephalitis (GAE). Cyst morphology was commonly used in the identification of *Acanthamoeba* at the subgenus level. A more accurate and consistent method based on the analysis of complete sequences of nuclear small ribosomal subunit RNA genes (*Rns*) has been developed. To date, no *Acanthamoeba* genotype identification and distribution in the Philippines has been reported. In this study, the ASA.S1 region of the *Acanthamoeba* sp. *Rns* was sequenced from 17 samples isolated from soil, water, and contact lens storage cases from different regions of the Philippines. Genotypes of the isolates were identified using BLAST and their phylogenetic positions relative to known Acanthamoeba isolates were determined using the model based (GTR+Γ) neighbor-joining, maximum likelihood and Bayesian inference analyses and the non-model based maximum parsimony analysis. Results show that the genotypes T5 and T4 are commonly found in soil, water, and contact lens storage case samples from different parts of the Philippines. Apart from these, a contact lens case isolate was identified as genotype T15.

Keywords: *Acanthamoeba, Rns* genotype, subgenus classification, phylogenetics, Philippines

BSD-36

THE SMALL SUBUNIT (SSU) RIBOSOMAL RNA GENE AS A GENETIC MARKER FOR IDENTIFYING INFECTIVE 3RD JUVENILE STAGE *Angiostrongylus cantonensis* IN GARDEN SLUGS

Ian Kendrich Fontanilla[1,2*] and Christopher Mark Wade[1]

[1]Institute of Genetics, Queen's Medical Centre
University of Nottingham, Nottingham NG7 2UH, United Kingdom
[2]Institute of Biology, College of Science
University of the Philippines, Diliman 1101, Quezon City, Philippines
E-mail: *ianfontanilla@hotmail.com

Angiostrongylus cantonensis is a strongylid nematode parasite that utilizes a variety of gastropods as intermediate hosts and the rat as final host. Its third juvenile stage can accidentally infect humans and cause eosinophilic meningoencephalitis. Accurately identifying the juvenile *A. cantonensis* in gastropod intermediate hosts can aid in controlling the disease the nematode causes. Here we have developed a molecular method using PCR-direct sequencing to identify the infective 3rd juvenile stage of *A. cantonensis*. We demonstrate that the 5' end of the small subunit (SSU) ribosomal (r) RNA gene is a suitable marker to identify *A. cantonensis* and distinguish it from other closely related *Angiostrongylus* species. When the SSU rRNA marker was employed on nematode populations extracted from the black slug *Laevicaulis alte* collected from 2 test sites in Quezon City, Philippines, the juvenile *A. cantonensis* was detected without difficulty. Other nematode species were also extracted from the slugs, and their phylogenetic position was also determined using the same SSU rRNA marker. The molecular technique developed in this study provides a rapid and accurate method for the identification of *A. cantonensis* when morphological identification proves difficult or inadequate.

Keywords: *Angiostrongylus cantonensis*, infective 3rd juvenile stage, SSU rRNA gene marker, *Laevicaulis alte*, molecular identification, eosinophiolic meningoenecphalitis

BSD-37

ISOLATION OF ACETYLCHOLINESTERASE INHIBITING PEPTIDES FROM CONUS MUSTELINUS VENOM

Anna Carlissa P. Arriola and Mary Rose M. Maballo*

Department of Biology, University of the Philippines Manila
Home address: 1528 F. Agoncillo St., Ermita, Manila
Tel.: (02)524-7971/(0922)6529603; E-mail: carlyarriola@yahoo.com

Venom from *Conus* is known to contain peptides which inhibit the function of ion channels and neurotransmitter receptors, but an effort to evaluate its inhibition against acetylcholinesterase (AchE) has yet to be conducted. Through Ellman's calorimetric method and Mus musculus bioassay, the crude venom extract and purified venom fractions from *Conus mustelinus* were investigated for in vivo and in vitro AchE inhibition activities. The crude extract was found to have significant AchE inhibitory activity (28.9% in vivo and 54.1% in vitro) and of the two peptide fractions isolated, Potential AchE Inhibiting Fraction-10 (PAIF-10, eluted at 21.15% B90) was also found to significantly inhibit AchE (30.5% in vivo and 44.8% in vitro), however no significant activity against AchE was observed from PAIF-11 (eluted at 20.68% B90) which inhibited it by 8.9% in vivo and -4.9% in vitro. Inhibition of AchE activity by *Conus* venom thus provides a promising approach to drug formulation.

Keywords: acetylcholinesterase, *conus*

BSD-38

MOLECULAR IDENTIFICATION OF *CRYPTOSPORIDIUM* SPP. IN ANIMAL HOSTS IN THE PHILIPPINES

Windell L. Rivera[1,2*] and John Anthony D.L. Yason[1,2]

[1]Institute of Biology, College of Science
[2]Molecular Protozoology Laboratory, Natural Sciences Research Institute
University of the Philippines Diliman, Quezon City
Telefax: 920-5471; E-mail: wlrivera@science.upd.edu.ph

Cryptosporidium spp. are coccidian parasites that are known to infect human and animal hosts. These pathogens are among the significant causes of gastrointestinal infection in many countries today. They have worldwide distributionand their infective stage (oocyst) is ubiquitous in the environment.

In the Philippines, information on *Cryptosporidium* is limited. In this study, we collected fecal samples from different known animal hosts of *Cryptosporidium*. We detected *Cryptosporidium* spp. using microscopy and a polymerase chain reaction (PCR) assay utilizing the organism's heat-shock protein gene. Pigs showed the highest rate (34.29%) of shedding *Cryptosporidium* oocysts followed by calves (20.41%) and chickens (5.66%). This study adds pigs and chickens as hosts of the pathogen in the country. In addition, we sequenced DNA fragments of the SSU rRNA gene to genotype isolates from calf stools. All the isolates were *C. parvum* except one, which was *C. canis*. This is the first report of genotyping of *Cryptosporidium* spp. in the Philippines.

Keywords: *Cryptosporidium*, genotype, heat shock protein gene, SSU rRNA gene, PCR, Philippines

BSD-39

GARLIC, GINGER AND ONION AS IMMUNOSTIMULANTS: A PRELIMINARY STUDY ON THE SPECIFIC AND NON-SPECIFIC IMMUNE RESPONSE OF AFRICAN CATFISH *CLARIAS GARIEPINUS* FINGERLINGS

Francis N. Baleta[1,2*], Patricia M. Candelaria[1,3], Godofredo P. Lubat[1,4], Jr. and Marlyn B. Llameg[1,4]

[1]Institute of Aquaculture, College of Fisheries and Ocean Sciences
University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines
[2]College of Agriculture and Fisheries,
Isabela State University Roxas, Roxas, Isabela
[3]Partido State University Lagonoy Campus, Lagonoy, Camarines Sur
[4]Southern Philippines Agri-business and Marine and Aquatic School of Technology Malita, Davao del Sur
Mobile: +63928-748-05-74; E-mail: fnbaleta19@yahoo.com

A preliminary study on the effects of garlic *Allium sativum*, ginger *Zingiber officinale* and onion *Allium cepa* as dietary immunostimulants on the specific and non-specific immune response of African catfish *Clarias gariepinus* was conducted. Catfish fingerlings (1.45±0.23) were fed with experimental diets for six weeks. After the feeding experiment, blood was taken from the caudal region of anaesthetized fish and hematocrit, total hemocyte count, total immunoglobulin and superoxide anion production were determined. Garlic-fed fish had the highest hematocrit (37.00 % ± 3.19) while the onion-fed group revealed the highest level (1.007 mg/ml) of immunoglobulin. On the other hand, the incorporation of ginger

in the diet elevated the production of superoxide anion (1.018 OD at 540nm). However, no difference were found on the levels of hematocrit, total immunoglobulin and superoxide anion production between the control and the experimental groups (P>0.05). The total hemocyte count showed significant difference between the control (10.37 ± 1.96) and onion (17.18 ± 1.72) fed group, but no significant difference with garlic (14.68 ± 1.0 and ginger (12.75 ± 1.20) fed group. The present study revealed that the incorporation of natural immunostimulants like garlic, ginger and onion could possibly increase the immunocompetence of the fish, and hence recommended to be tested for fish culture. The effects of age of fish, duration of feeding and the amount of immunostimulant to be used are to be investigated.

Keywords: African catfish, local immunostimulants, immune response, dietary immunostimulation, immunological parameters

BSD-40

THE EFFECT OF Cry 1Ab PROTEIN ON THE GREAT EGG FLY, Hypolimnas bolina philippensis (Linnaeus): A NON-TARGET LEPIDOPTERA

Bonifacio F. Cayabyab[1], Edwin P. Alcantara[2], Augusto C. Sumalde[1], Wilma R. Cuaterno[3], Blair D. Siegfried[3] and Josemarie M. Belen[1]

[1]Crop Protection Cluster College of Agriculture,
Email: bfcayabyab@yahoo.com
[2]National Institute of Molecular Biology and Biotechnology
University of the Philippine Los Baños, College, Laguna 4031
[3]Crop Protection Division
Bureau of Plant Industry, Malate, Manila,
[4]Institute of Agriculture and Natural Resources
Department of Entomology University of Nebraska
Lincoln, NE 830816

This study was conducted from January 2007 August 2007 at NCPC CPC UP Los Baños to determine if ingestion of Cry1Ab protein is a hazard to Hypolimnas bolina philippensis (Linnaeus) larvae since it is most likely exposed to Bt corn pollen in the field.

Bioassay tests through leaf dip method were conducted using 10x the maximum Cry 1Ab protein concentration found in Bt corn pollen (0.09mg/g pollen). Three days old larvae were individually exposed to 1 cm diameter leaf disc of Ipomoea triloba Linnaeus soaked in solution of Cry1Ab protein. Controls were individually exposed to I. triloba leaf discs soaked in 0.1 M Carbonate buffer. Fifty larvae each were used in treated and control experiments, respectively.

Nine trials or 450 treated larvae and 450 non-treated larvae were utilized. A total of 30 larvae died on the treated larvae while 25 perished in the control. The larval mean percent mortality and weight of treated and control trials were statistically compared using t-test. The difference in mortality of the treated and control means were not significant (P = 0.536). The difference in weight between treated and control groups of larvae were also not significant (Pd.f= 16 = 0.692).

Although many Lepidopterous species including butterflies are known to be sensitive to Cry1Ab protein (Wolf et al., 2003), the effect of Cry1Ab protein on the butterfly, Hypolimnas bolina philippensis (Linnaeus) larvae was negligible in terms of larval mortality and weight. These results suggests the absence of receptor molecules for Cry1Ab protein in the guts of H. bolina larvae. Both cadherins and amino peptidases have been identified as putative receptor molecules for Cry1Ab proteins in other Lepidopterans (Ferré and van Rie et al., 2002).

Keywords: Cry 1Ab protein, Non-target Lepidoptera, Hypolimnas bolina philippensis, susceptibility, t-test, larvae

BSD-41

PACHYRRHYNCHINE WEEVILS (COLEOPTERA: CURCULIONIDAE) OF POLILLO ISLAND, QUEZON PROVINCE, PHILIPPINES

Sheryl A. Yap[1]* and Ireneo L. Lit, Jr.[1,2]

[1]Entomological Museum, Museum of Natural History
[2]Institute of Biological Sciences, College of Arts and Sciences
University of the Philippines Los Baños, College, Laguna
Tel.: 049 536 2864; *E-mail: uplbmnh@yahoo.com.ph

Pachyrrhynchine weevils belong to the Order Coleoptera, Family Curculionidae. They are endemic to the Philippines and can be found in less explored mountainous regions between 500 to 2000 meters above sea level. Their habitats consist of vast tropical vegetation, open, mixed forests with dense undergrowth along rivers and ravines or on ridges and mountains, with most species living on smaller trees, bushes, shrubs, or ferns.

Polillo Island was selected as one of the sampling sites for the ongoing taxonomic study of the group as well as a contribution to the study of arthropod biodiversity of the island group. Insect collections were done by hand picking and the use of insect nets and beating sheets. Collected specimens were pinned and identified. From the available collections, twelve (12) species comprising three genera, *Homalocyrtus, Metapocyrtus* and *Pachyrrhynchus* were determined. One (1) species belongs to the genus *Homalocyrtus*, six (6) to the *Metapocyrtus*, and 4 to *Pachyrrhynchus*. They were mostly from the remnant primary forest of the Sibulan Watershed, Pinaglubayan in the town of Polillo and and some secondary growths in

in the town of Burdeos.

Keywords: Curculionidae, *Homalocyrtus*, *Metapocyrtus*, *Pachyrrhynchus*, weevil

BSD-42

BOOM AND BUST TRENDS AND OTHER UPDATES AMONG LOCAL PHILIPPINE POPULATIONS OF THE BUFF COCONUT MEALYBUG, *NIPAECOCCUS NIPAE* (MASKELL) (HEMIPTERA: PSEUDOCOCCIDAE)

Merdelyn T. Caasi-Lit[1], Ireneo L. Lit, Jr.[2*] and Ariel R. Larona[3]

[1] Entomology Laboratory, Institute of Plant Breeding,
Crop Science Cluster, College of Agriculture
[2] Institute of Biological Sciences, College of Arts and Sciences and
Entomological Museum, Museum of Natural History
[3] Forestry Herbarium, Museum of Natural History
University of the Philippines Los Baños, College, Laguna, Philippines
*Email: mtcaasi_lit@yahoo.com, illit_jr@yahoo.com

The buff coconut mealybug, Nipaecoccus nipae (Maskell), was introduced into the country circa 2003. Its penultimate origin is unknown and the local distribution data were last recorded in Southern Tagalog provinces of Batangas, Cavite, Laguna and Quezon, and in Davao City. Since its introduction, it has devastated the atis (Annona squamosa L.) industry of Lobo, Batangas and seriously affected the tuba yield in lambanog-producing areas of Laguna and Quezon. The results of our population monitoring activities in Los Baños, Laguna and surrounding areas from 1999-2007 are presented. Population booms during the dry months or long periods without rain and sudden declines after the first few weeks of the rainy season have characterized the trends in the local population levels. However, the occurrence of supertyphoon 'Milenyo' on September 28, 2006 with more than 400 cm rainfall and accompanying strong winds severely affected the local mealybug population such that they have, so far, been limited to small colonies. This does not preclude, nevertheless, the possibility of resurgence when another long period without rain comes. New host plant and locality records are also herein reported, bringing the total number of locally available host plants to 60 species and the Philippine distribution spreading to some parts of Northern, Central and Southern Luzon, Visayas and Mindanao. A few local natural enemies like the brown lacewing (Hemerobiidae) have now been observed but their biology, efficiency and predatory capacity have yet to be studied.

Keywords: buff coconut mealybug, *Nipaecoccus nipae*, Pseudococcidae, invasive species

BSD-43

BUTTERFLIES (RHOPALOCERA) OF POLILLO ISLAND, QUEZON PROVINCE, PHILIPPINES

Ireneo L. Lit, Jr.

Institute of Biological Sciences, College of Arts and Sciences and
Entomological Museum, Museum of Natural History, OVCRE
University of the Philippines Los Baños, College, Laguna, Philippines
E-mail: illit_jr@yahoo.com

The diversity of arthropods of Polillo Island are currently being studied as a contribution to the documentation of the overall biological diversity of the Polillo group of islands as well as the first of projected series of studies of terrestrial arthropod biodiversity in conservation priority areas in the Philippines. This particular portion of the research project focuses on the diversity of butterflies (Lepidoptera: Papilionoidea and Hesperioidea). A total of 117 individuals were observed and/or collected. These belong to 42 species, distributed in seven families, with the family Nymphalidae as the best represented group in terms of number of species and individuals. Papilionidae or the swallowtails, which included three that belong to the birdwing tribe, came next with seven species followed by the Pieridae with six species. In terms of number of individuals, however, pierids were superior to the papilionids. Preliminary analysis indicate a diversity value of (Shannon Index) H = 3.480 and e = 0.912. For a relatively small area, and with the limited time to observe, such number of species and diversity index are remarkably high.

The most common was the yellow pierid *Eurema hecabe* followed by an undetermined species of lycaenid belonging to *Jamides*. The common birdwing, *Troides rhadamantus,* listed together with the other *Troides* and *Trogonoptera* in CITES Appendix II, can still be found around the edges of the Sibulan watershed and the surrounding thickets of the agricultural areas of Pinaglubayan. The same is true for another endemic butterfly, *Pachliopta mariae almae.* The host plants of these butterflies in the surrounding areas still need to be investigated.

Keywords: butterflies, Rhopalocera, Papilionoidea, Hesperioidea, terrestrial arthropod biodiversity, Polillo island.

BSD-44

EFFECTS OF *Chlorella* sp. and *Spirulina* sp. ON SURVIVAL, GROWTH, AND RESISTANCE OF *Penaeus monodon* Larvae AGAINST *Vibrio harveyi*

Angeli A. Valera, Karyl Marie B. Fabricante* and Buenaflor D. Jimenez*

Department of Biological Sciences, CSM
MSU-Iligan Institute of Technology, Iligan City
E-mail: angeli_valera@yahoo.com; 09177230713

The study is focused on the effects of *Chlorella* sp. and *Spirulina* sp. on survival, growth, and resistance of *Penaeus monodon* larvae against *Vibrio harveyi*. Two set-ups with five treatments in three replicates were tested. Set-up A consists of *Skeletonema*:*Chlorella* (*Sk*:*Ch*) treatment ratios while set-up B consists of *Skeletonema*:*Spirulina* (*Sk*:*Sp*) treatment ratios. Feeding was initiated at zoea first day (Z_1) stage until the postlarval fifth day (PL_5) stage. Survival and growth were determined at mysis first day (M_1) and postlarval fifth day (PL_5) stages. Growth was measured at M_1 and PL_5. Resistance of larvae was determined in terms of survival at PL_5 after a challenge dose (1.5×10^3 cells/ml) of *V. harveyi* at M_1.

Treatments with *Chlorella*:*Skeletonema* ratios showed higher percentage survival, growth, and resistance compared to those with pure *Chlorella* sp. and *Skeletonema* sp. Treatments with *Spirulina* sp. showed lower survival and growth but higher resistance.

The results suggest that *Chlorella* sp. can enhance *P.monodon* survival, growth, and resistance. It also has a potential to replace *Skeletonema* as food for *P. monodon* in the earlier stages. *Spirulina* sp. has no significant role in increasing the growth and survival of *P. monodon*, but play a role in resistance against *V. harveyi*.

Keywords: *Chlorella* sp., *Spirulina* sp., *Penaeus monodon*, *Vibrio harveyi*, prawn industry

BSD-45

A COMPARATIVE STUDY ON THE SUSCEPTIBILITY OF THREE ERYTHRINA SPECIES TO ERYTHRINA GALL WASP (*Quadrastichus erythrinae* K.)

Josephine Espinoza Tondo and Abercio Rotor*

Graduate School, University of Santo Tomas, Manila and
University of Regina Carmeli, Catmon, Malolos City, Bulacan
Tel.: 044-791-72-02/044-791-02-72; Mobile: 09193657282;
E-mail: joswilshen25@yahoo.com

The study characterized gall infestation of the two species and a variety of *Erythrina* trees bya tiny wasp, *Quadrastichus erythrinae* Kim, which lives in the soft tissues of young leaves and petioles, stimulating gall formation leading to defoliation, dieback and death. All of the species and a variety studied were susceptible to the pest, with *Erythrina variegata* var *orientalis* as the most susceptible, followed by *Erythrina variegata* and *Erythrina crista-galli* as the least susceptible. Average gall size in *E.variegata* var *orientalis* is 10.27mm on petiole and 5.84mm on the leaf; *E. variegata*, 9.87mm and 5.81mm respectively; and in *E. crista-galli*, 5.72 mm and 3.34 mm respectively. All four test plants of *E.variegata* var *orientalis* succumbed to the pest after 90 days; while *E. variegata* remained heavily infested but alive to the end of the experiment. Two of the four test plants of *E. crista-galli* showed light infestation, while the other two were not infested. The egg, larva and pupa stages of *Q. erythrinae* K. are spent inside the gall, ensconced in a chamber as the gall increases progressively in size or coalesce with adjacent galls Phytochemcial analyses showed no differences among infested species and a variety of *Erythrina*. Studies on surviving *Erythrina* trees found in the open are recommended with the end in view of obtaining resistant propagating materials on one hand, while studies on natural enemies of *Q. erythrinae* K. be conducted on the other.

Keywords: *Erythrina*,*Quadrastichus*,gall, phytochemical analyses,infestation

BSD-46

IDENTIFICATION OF INDICATOR SPECIES FROM ARTHROPOD COMMUNITIES USING PRINCIPAL RESPONSE CURVE ANALYSIS

Edwin P. Alcantara[1]* and Ma. Dulce J. Mostoles[2]

[1]National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Baños, College, Laguna
[2]Camarines Sur State Colleges, Pili, Camarines Sur
Email: alcantara12000@yahoo.com

Monitoring for secondary ecological effects is part of the responsible stewardship of transgenic crops. Since it is practically impossible to study the response of all the non-target organisms in an arthropod community found in a transgenic crop ecosystem, the use of indicator species for the purpose of monitoring is recommended. The present study was conducted to identify an indicator arthropod species in Bt corn using principal response curve (PRC) analysis. The method was tested by analyzing abundance data from samples taken from arthropod communities in 3 commercial Bt corn farms. An equal number of farms planted with near isoline non-Bt corn served as controls. Affiliation of specific arthropod species and functional groups was identified using ordination plot produced by redundancy (RDA) analysis. Results of the PRC analysis revealed the highest species weight (0.57) for predatory coccinellid beetle, *Micraspis discolor*. RDA ordination plot also showed that *M. discolor* is highly associated with Bt corn. There was no specific functional group association to either Bt- or non-Bt corn. However, there were other predators associated with Bt corn such as the lady beetle *Chilomones sexmaculatus*, the black cricket *Metioche vittaticolis* and spiders. Among the parasitoids, ichneumonids were highly associated with Bt corn. Braconids and scelionids were affiliated with non-Bt corn. The phytophagous silk beetle *Monolepta bifasciata* and the homopteran *Chanitus* sp. were also highly associated with Bt corn. There was no significant difference (P=0.452) in arthropod composition between Bt- and non-Bt corn farms. In conclusion, *Micraspis discolor* could be used as indicator species because it will most likely follow overall arthropod community response to Bt corn.

Keywords: indicator species, *Micraspis discolor*, principal response curve analysis, redundancy analysis, Bt corn

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Edwin P. Alcantara[1]* and Ma. Dulce J. Mostoles[2]

[1]National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Baños, College, Laguna
[2]Camarines Sur State Colleges, Pili, Camarines Sur
Email: alcantara12000@yahoo.com

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Keywords: indicator species, *Micraspis discolor*, principal response curve analysis, redundancy analysis, Bt corn

BSD-48

MARINE-DERIVED FUNGI FROM MANILA BAY, CAVITE AND BATANGAS AS SOURCES OF ANTIMICROBIAL METABOLITES

Marc Louie C. Yao[1], James Daniel H. Villanueva[1], Michelle Love S. Tumana[1], Joann G. Calimag[1] and Thomas Edison E. dela Cruz[1,2*]

[1] Department of Biological Sciences
[2] Research Center for the Natural Sciences
University of Santo Tomas
España 1015. Manila, Philippines
Tel.: +632 406-1611 local 4043; Fax no.: +632 731-4031
*E-mail: tedelacruz@mnl.ust.edu.ph

Several marine *Vibrio* species may cause diseases and damages to animals in aquaculture and to seaweeds in seaweed farms. If not controlled, such infection may result to great economic losses. Fungi from the marine environment could be potential sources of secondary metabolites active against species of *Vibrio*. Thus, our research study aims to isolate marine fungi from seawater and marine sediments and test their inhibitory activities against marine bacteria and standard test microorganisms. Seawater and marine sediments were collected from Manila Bay and nearby surrounding coastal provinces, Cavite and Batangas. Collected samples were spread-plated on Malt Extract Agar (MEAS) supplemented with 33 g/L marine salts, 500 mg/L tetracycline and 300 mg/L streptomycin. Following incubation, we have isolated 74 fungal strains, fourteen of which were grown on MEAS for the production of secondary metabolites and on PDA with or without marine salts for morphocultural characterization. Crude culture filtrates were then tested for their inhibitory activities against pathogenic marine bacteria and other test microorganisms. Metabolites present in the crude culture extracts were detected with different spray reagents using thin layer chromatography. Morphocultural characterization identified most of the isolates as *mycelia sterila*, as the isolates did not produce spores in culture. Several fungal strains grew better in the presence of marine salts. Results also showed that all isolates were active against at least one of the test organisms, e.g. *Vibrio fisheri*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Mycobacterium phlei*, and *Fusarium oxysporum*. None showed activity against *Vibrio harveyi* and *Escherichia coli*. Finally, detection of secondary metabolites using TLC showed the presence of steroids, sterols and triterpenes on the crude culture extracts.

Keywords: marine fungi, secondary metabolites, antimicrobial activities, bioassay

BSD-49

DRYING BEHAVIOR OF PORPHYRA (GAMET) FROM ABLAN, BURGOS ILOCOS NORTE, NORTHERN PHILIPPINES USING CONVECTION OVEN

Zenaida M Agngarayngay*, Loecelie B. Taclan and Ana Fe C. Llaguno

Mariano Marcos State University
City of Batac 2906, Ilocos Norte
Tel.: (077)792-313; Telefax: (077)792-2547
*E-mail: zeny_950@yahoo.com; rddirectorate@yahoo.com

This study was conducted to determine the drying behavior of *Porphyra* locally known as "gamet using convection oven as sun drying is the only post harvest practice employed by the gatherers in Ilocos Norte to prolong its shelf life.

Samples of *Porphyra* were gathered from the supra littoral zone of Burgos, Ilocos Norte. Drying curves were used to explain the relationship between moisture content (MC), drying rate and time. The initial MC of the samples was taken prior to the drying process and every hour thereafter until constant weight of samples was attained. The moisture reduction per hour was determined by considering the weight reduction of the samples per unit hour. Three samples were prepared for every temperature level. Drying was done by batch. Each batch was subjected to certain drying temperature (DT). The drying temperatures were 40 °C, 50 °C and 60 °C. Every hour the samples were taken out from the drying oven, placed in a dessicator for ten minutes before taking the weight loss. Drying was done until the weight of the samples was constant for at least two consecutive readings.

Results showed that *Porphyra* has reduced MC from 38% to 17%. Drying time and moisture loss was highly significant with R^2= 0.97 of samples dried at 40 °C. The data were fitted to the exponential model. This model proved to have good prediction capability on oven dried *Porphyra*.

The study proved that the higher the DT, the higher is the rate of MC reduction. Conversely, the lower the DT, the higher is the correlation coefficient. These results imply that *Porphyra* should be dried at 40 °C for safer storage and better product quality for a longer shelf life than those dried under the sun.

Keywords: *Porphyra*, supra-littoral zone, convection oven, moisture content, drying behavior, drying curve

BSD-50

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

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

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

Somatic growth (wet weight and equatorial and polar test diameters), gonad growth (gonadosomatic index) and gonad quality (color and granularity) of the sea urchin *T. gratilla* fed prepared diets were studied *in vitro* using plastic basins from February to June 2006. The study consisted of three treatments, replicated thrice and arranged in CRD as follows: I-Fresh *Sargassum sp.* (control), II-Dried pellets, and III-Fresh Extruded pellets. The dried and fresh extruded pellets were mainly of *Sargassum sp.* With 6.0% binder (corn starch and gelatin).

No significant variations were observed in the somatic growth of *T. gratilla* among the different feeding treatments. 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 (p0.05). First spawning was observed at about 1.5 culture months. Observed water parameters were within the favorable ranges for growth and survival of *T. gratilla.*

The successful introduction of prepared diets fro *T. gratilla* opens the possibility of incorporating gonad color enhancers such as carotenoids in the diet that improved the quality of the organism for market consumption. This necessitates further studies specifically using locally available pigment sources like tomato and squash. The study likewise demonstrated the viability of land-based culture of the organism using both the fresh natural food and prepared diets. This will be important in sustaining a year-round harvest and possible broodstock source for hatchery and seed stock production.

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

BSD-51

STOCK ASSESSMENT OF SHALLOW WATER HOLOTHURIA IN CAMOTES ISLANDS, CEBU, PHILIPPINES: BASIS FOR A PROPOSED CONSERVATION AND MANAGEMENT PLAN

Serapion N. Tanduyan*, Panfilo E. Ciriaco, Ricardo B. Gonzaga, Wilfredo G. Anoos, Lourdes M. Garciano and Berenice T. Andriano

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

Sea cucumbers of Camotes Islands were studied. Thirty-six coastal barangays were assessed,10 in Poro; 10 in San Francisco; eleven in Pilar and five in Tudela, Cebu. Day and night assessments were done using transect-quadrat method Three transects were laid in the tidal area of each barangay up to 3m depth of water. Meristic and morphometric measurements and identification of the collected Holothuria and other associated species were done.

Results showed that there were twenty species of holothuria found throughout Camotes Islands and 16 were identified and 4 were unidentified. *Bat Pisot* (unidentified Holothuria) has the highest frequency of 250 pieces during the day assessment and Bat Marcos (*Stichopus hermanni*) during the night assessment with 18 pieces. *Pearsonothuria graeffei* has the lowest frequency (1) during day assessment and *Actinopyga lecanora, Holothuria atra, Pearsonothuria graeffei* and Bat Otan-otan during night assessment (1).

Holothuria pulla has the highest weight (175-225 grams) both in day and night assessment. *Euapta godeffroyi* has the lowest in weight (13 g.) during the day assessment and *Actinopyga lecanora* is lowest during the night assessment (8g). The species which ranked first for the highest in length is *Euapta godeffroyi* (30-43 cm) both night and day assessment and the lowest is *Holothuria nobilis* (6cm).

Results further showed that there are 13 common species of holothuria found in the four municipalities which are: *Bohadschia marmorata, Holothuria pulla, Holothuria scabra, Holothuria impatiens, Stichopus hermanni, Euapta godeffroyi, Bohadschia paradoxa, Stichopus horrens, Balat kagiron, Balat Langgi-langgi, Holothuria fuscopunctata, Stichopus variegatus and Stichopus sp.*

The most diverse municipality is San Francisco; Cebu which has 18 species followed by Poro (15); Pilar (14) and Tudela (13). For the distinct species, *Holothuria rigida* is found only in San Francisco, Cebu followed by *Pearsonothuria graffei* (in Tudela and Pilar);

Keywords: Stock Assessment, *Holothuria*, Camotes Islands, Conservation

BSD-52

ANURAN SPECIES COMPOSITION, DISTRIBUTION, AND CONSERVATION STATUS IN MT. SAMBILIKAN, DIWATA RANGE, AGUSAN DEL SUR

Karyl Marie B. Fabricante and Olga M. Nuñeza*

Department of Biological Sciences
Mindanao State University-Iligan Institute of Technology
E-mail: Karyl_marie@yahoo.com; 09177190882

Mt. Sambilikan, Diwata range is a part of the country's key conservation sites and Important Bird Areas (IBA). Diversity, distribution including microhabitat preferences, and conservation status of its anurans, being biological indicators of disturbance, were determined. The area was assessed using visual encounter survey and pitfall trapping from October 21 to November 22, 2006 in three elevation gradients (670-1,050masl) with corresponding vegetation types: mixed lowland dipterocarp (1), mixed primary-secondary (2), and upper montane-mossy forest (3). Diet and endoparasite of the endemic *Rana grandocula* and non-endemic *Limnonectes magnus* were noted through digestive tract examination of voucher specimens.

Twenty-one species (four families and 14 genera) were recorded with 12 (57%) endemic and eight (38%) threatened. Highest species richness (R=15), species diversity (H'=2.409), and evenness (E=0.742) in site 2 confirm disturbance and altitude to limit frog species distribution. Microhabitat preferences revealed that majority of the anurans were terrestrial, burrowers, or amphibious species (type IV). Three species were found to be economically important as food. Diet and endoparasite examination of *L. magnus* and *R. grandocula* depict the frogs' high dependence on invertebrate fauna especially of the order Orthoptera, niche overlap, possible competition between endemic and non-endemic species, and the importance of both species as intermediate hosts to cestodes and nematodes. Unsustainable hunting and habitat loss through conversion of forest fragments into abaca and Falcata plantations appear to be the major threats in the area.

Mt. Sambilikan although disturbed, still harbors forest-dependent, endemic, and threatened anuran species. Unfortunately, a high probability of risk exists if present rates of forest destruction and unsustainable community practices continue. A pressing need to create holistic conservation mechanisms, with the involvement of the local community, to effectively protect and possibly connect last fragments of habitats of the local biodiversity, is crucial for the survival of these endemic and threatened species.

Keywords: anuran diversity, environmental health, biodiversity loss, conservation

BSD-43

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Ireneo L. Lit, Jr.

Institute of Biological Sciences, College of Arts and Sciences and
Entomological Museum, Museum of Natural History, OVCRE
University of the Philippines Los Baños, College, Laguna, Philippines
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BSD-44

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Keywords: *Chlorella* sp., *Spirulina* sp., *Penaeus monodon*, *Vibrio harveyi*, prawn industry

BSD-45

A COMPARATIVE STUDY ON THE SUSCEPTIBILITY OF THREE ERYTHRINA SPECIES TO ERYTHRINA GALL WASP (*Quadrastichus erythrinae* K.)

Josephine Espinoza Tondo and Abercio Rotor*

Graduate School, University of Santo Tomas, Manila and
University of Regina Carmeli, Catmon, Malolos City, Bulacan
Tel.: 044-791-72-02/044-791-02-72; Mobile: 09193657282;
E-mail: joswilshen25@yahoo.com

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BSD-46

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Edwin P. Alcantara[1]* and Ma. Dulce J. Mostoles[2]

[1]National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Baños, College, Laguna
[2]Camarines Sur State Colleges, Pili, Camarines Sur
Email: alcantara12000@yahoo.com

Monitoring for secondary ecological effects is part of the responsible stewardship of transgenic crops. Since it is practically impossible to study the response of all the non-target organisms in an arthropod community found in a transgenic crop ecosystem, the use of indicator species for the purpose of monitoring is recommended. The present study was conducted to identify an indicator arthropod species in Bt corn using principal response curve (PRC) analysis. The method was tested by analyzing abundance data from samples taken from arthropod communities in 3 commercial Bt corn farms. An equal number of farms planted with near isoline non-Bt corn served as controls. Affiliation of specific arthropod species and functional groups was identified using ordination plot produced by redundancy (RDA) analysis. Results of the PRC analysis revealed the highest species weight (0.57) for predatory coccinellid beetle, *Micraspis discolor*. RDA ordination plot also showed that *M. discolor* is highly associated with Bt corn. There was no specific functional group association to either Bt- or non-Bt corn. However, there were other predators associated with Bt corn such as the lady beetle *Chilomones sexmaculatus*, the black cricket *Metioche vittaticolis* and spiders. Among the parasitoids, ichneumonids were highly associated with Bt corn. Braconids and scelionids were affiliated with non-Bt corn. The phytophagous silk beetle *Monolepta bifasciata* and the homopteran *Chanitus* sp. were also highly associated with Bt corn. There was no significant difference (P=0.452) in arthropod composition between Bt- and non-Bt corn farms. In conclusion, *Micraspis discolor* could be used as indicator species because it will most likely follow overall arthropod community response to Bt corn.

Keywords: indicator species, *Micraspis discolor*, principal response curve analysis, redundancy analysis, Bt corn

BSD-47

CLIMATE CHANGE IMPACT ON THE PEST STATUS OF SMALL ISLANDS: THE FUGA ISLAND EXPERIENCE

Bonifacio F. Cayabyab[1], Pablito G. Gonzales[1], Melvin D. Ebuenga[1] and Wilma R. Cuaterno[2]

[1]Crop Protection Cluster, National Crop Protection Center
University of the Philippines Los Baños, College, Laguna 4031
Telefax 63 49 536 2409; Email: bfcayabyab@yahoo.com
[2] Crop Protection Division, Bureau of Plant Industry
San Andres Manila

Climate change can greatly affect the pest status of small islands such as Fuga Island in the Babuyan Channel in Northern Luzon. The lack of rainfall from November 2006 led to an outbreak of migratory locust, Locusta migratoria manilensis Meyen on January 2007 in the island. Rice and corn were attacked. Later a similar population build-up occurred in July 2007. We did a volunteer work in this island on July 31 up to August 2, 2007 thru lectures and field practicum on how to cope up with changing pest status not only of explosive pest like the migratory locust but also the other pest that attack their crops. We also brought with us the green muscardine fungi, Metarhizium anisopliae to check the new population build up. This condition in Fuga Island is a repeat of the aftermath of Mt. Pinatubo eruption. There were few remaining green vegetations during that time due to ash fall. This favorable environment which is akin to the effect of drought brought about by the climate change favors the rapid population build up of explosive pest like the migratory locust in Fuga Island. We also observed this phenomenon from 1998-2003 in the islands of Masbate, Romblon, Negros and also in La Union last year.

It is imperative that mitigating measures should be formulated now together with the affected populace particularly in resource challenged islands like the Fuga Island. Early warning system should be put in place for droughts or even extreme rainfalls. Islanders should likewise be informed on the detection and management of explosive pests during dry and warm periods due to climate change.

Keywords: Climate change, Fuga Island, migratory locust, pest, small islands, Mt. Pinatubo, drought.

BSD-48

MARINE-DERIVED FUNGI FROM MANILA BAY, CAVITE AND BATANGAS AS SOURCES OF ANTIMICROBIAL METABOLITES

Marc Louie C. Yao[1], James Daniel H. Villanueva[1], Michelle Love S. Tumana[1], Joann G. Calimag[1] and Thomas Edison E. dela Cruz[1,2*]

[1] Department of Biological Sciences
[2] Research Center for the Natural Sciences
University of Santo Tomas
España 1015, Manila, Philippines
Tel.: +632 406-1611 local 4043; Fax no.: +632 731-4031
*E-mail: tedelacruz@mnl.ust.edu.ph

Several marine *Vibrio* species may cause diseases and damages to animals in aquaculture and to seaweeds in seaweed farms. If not controlled, such infection may result to great economic losses. Fungi from the marine environment could be potential sources of secondary metabolites active against species of *Vibrio*. Thus, our research study aims to isolate marine fungi from seawater and marine sediments and test their inhibitory activities against marine bacteria and standard test microorganisms. Seawater and marine sediments were collected from Manila Bay and nearby surrounding coastal provinces, Cavite and Batangas. Collected samples were spread-plated on Malt Extract Agar (MEAS) supplemented with 33 g/L marine salts, 500 mg/L tetracycline and 300 mg/L streptomycin. Following incubation, we have isolated 74 fungal strains, fourteen of which were grown on MEAS for the production of secondary metabolites and on PDA with or without marine salts for morphocultural characterization. Crude culture filtrates were then tested for their inhibitory activities against pathogenic marine bacteria and other test microorganisms. Metabolites present in the crude culture extracts were detected with different spray reagents using thin layer chromatography. Morphocultural characterization identified most of the isolates as *mycelia sterila*, as the isolates did not produce spores in culture. Several fungal strains grew better in the presence of marine salts. Results also showed that all isolates were active against at least one of the test organisms, e.g. *Vibrio fisheri*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Mycobacterium phlei*, and *Fusarium oxysporum*. None showed activity against *Vibrio harveyi* and *Escherichia coli*. Finally, detection of secondary metabolites using TLC showed the presence of steroids, sterols and triterpenes on the crude culture extracts.

Keywords: marine fungi, secondary metabolites, antimicrobial activities, bioassay

BSD-49

DRYING BEHAVIOR OF PORPHYRA (GAMET) FROM ABLAN, BURGOS ILOCOS NORTE, NORTHERN PHILIPPINES USING CONVECTION OVEN

Zenaida M Agngarayngay*, Loecelie B. Taclan and Ana Fe C. Llaguno

Mariano Marcos State University
City of Batac 2906, Ilocos Norte
Tel.: (077)792-313; Telefax: (077)792-2547
*E-mail: zeny_950@yahoo.com; rddirectorate@yahoo.com

This study was conducted to determine the drying behavior of *Porphyra* locally known as "gamet using convection oven as sun drying is the only post harvest practice employed by the gatherers in Ilocos Norte to prolong its shelf life.

Samples of *Porphyra* were gathered from the supra littoral zone of Burgos, Ilocos Norte. Drying curves were used to explain the relationship between moisture content (MC), drying rate and time. The initial MC of the samples was taken prior to the drying process and every hour thereafter until constant weight of samples was attained. The moisture reduction per hour was determined by considering the weight reduction of the samples per unit hour. Three samples were prepared for every temperature level. Drying was done by batch. Each batch was subjected to certain drying temperature (DT). The drying temperatures were 40 °C, 50 °C and 60 °C. Every hour the samples were taken out from the drying oven, placed in a dessicator for ten minutes before taking the weight loss. Drying was done until the weight of the samples was constant for at least two consecutive readings.

Results showed that *Porphyra* has reduced MC from 38% to 17%. Drying time and moisture loss was highly significant with R^2= 0.97 of samples dried at 40 °C. The data were fitted to the exponential model. This model proved to have good prediction capability on oven dried *Porphyra*.

The study proved that the higher the DT, the higher is the rate of MC reduction. Conversely, the lower the DT, the higher is the correlation coefficient. These results imply that *Porphyra* should be dried at 40 °C for safer storage and better product quality for a longer shelf life than those dried under the sun.

Keywords: *Porphyra*, supra-littoral zone, convection oven, moisture content, drying behavior, drying curve

BSD-50

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

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

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

Somatic growth (wet weight and equatorial and polar test diameters), gonad growth (gonadosomatic index) and gonad quality (color and granularity) of the sea urchin *T. gratilla* fed prepared diets were studied *in vitro* using plastic basins from February to June 2006. The study consisted of three treatments, replicated thrice and arranged in CRD as follows: I-Fresh *Sargassum sp.* (control), II-Dried pellets, and III-Fresh Extruded pellets. The dried and fresh extruded pellets were mainly of *Sargassum sp.* With 6.0% binder (corn starch and gelatin).

No significant variations were observed in the somatic growth of *T. gratilla* among the different feeding treatments. 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 (p0.05). First spawning was observed at about 1.5 culture months. Observed water parameters were within the favorable ranges for growth and survival of *T. gratilla.*

The successful introduction of prepared diets fro *T. gratilla* opens the possibility of incorporating gonad color enhancers such as carotenoids in the diet that improved the quality of the organism for market consumption. This necessitates further studies specifically using locally available pigment sources like tomato and squash. The study likewise demonstrated the viability of land-based culture of the organism using both the fresh natural food and prepared diets. This will be important in sustaining a year-round harvest and possible broodstock source for hatchery and seed stock production.

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

BSD-51

STOCK ASSESSMENT OF SHALLOW WATER HOLOTHURIA IN CAMOTES ISLANDS, CEBU, PHILIPPINES: BASIS FOR A PROPOSED CONSERVATION AND MANAGEMENT PLAN

Serapion N. Tanduyan*, Panfilo E. Ciriaco, Ricardo B. Gonzaga, Wilfredo G. Anoos, Lourdes M. Garciano and Berenice T. Andriano

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

Sea cucumbers of Camotes Islands were studied. Thirty-six coastal barangays were assessed,10 in Poro; 10 in San Francisco; eleven in Pilar and five in Tudela, Cebu. Day and night assessments were done using transect-quadrat method Three transects were laid in the tidal area of each barangay up to 3m depth of water. Meristic and morphometric measurements and identification of the collected Holothuria and other associated species were done.

Results showed that there were twenty species of holothuria found throughout Camotes Islands and 16 were identified and 4 were unidentified. *Bat Pisot* (unidentified Holothuria) has the highest frequency of 250 pieces during the day assessment and Bat Marcos (*Stichopus hermanni*) during the night assessment with 18 pieces. *Pearsonothuria graeffei* has the lowest frequency (1) during day assessment and *Actinopyga lecanora, Holothuria atra, Pearsonothuria graeffei* and Bat Otan-otan during night assessment (1).

Holothuria pulla has the highest weight (175-225 grams) both in day and night assessment. *Euapta godeffroyi* has the lowest in weight (13 g.) during the day assessment and *Actinopyga lecanora* is lowest during the night assessment (8g). The species which ranked first for the highest in length is *Euapta godeffroyi* (30-43 cm) both night and day assessment and the lowest is *Holothuria nobilis* (6cm).

Results further showed that there are 13 common species of holothuria found in the four municipalities which are: *Bohadschia marmorata, Holothuria pulla, Holothuria scabra, Holothuria impatiens, Stichopus hermanni, Euapta godeffroyi, Bohadschia paradoxa, Stichopus horrens, Balat kagiron, Balat Langgi-langgi, Holothuria fuscopunctata, Stichopus variegatus and Stichopus sp.*

The most diverse municipality is San Francisco; Cebu which has 18 species followed by Poro (15); Pilar (14) and Tudela (13). For the distinct species, *Holothuria rigida* is found only in San Francisco, Cebu followed by *Pearsonothuria graffei* (in Tudela and Pilar);

Keywords: Stock Assessment, *Holothuria*, Camotes Islands, Conservation

BSD-52

ANURAN SPECIES COMPOSITION, DISTRIBUTION, AND CONSERVATION STATUS IN MT. SAMBILIKAN, DIWATA RANGE, AGUSAN DEL SUR

Karyl Marie B. Fabricante and Olga M. Nuñeza*

Department of Biological Sciences
Mindanao State University-Iligan Institute of Technology
E-mail: Karyl_marie@yahoo.com; 09177190882

Mt. Sambilikan, Diwata range is a part of the country's key conservation sites and Important Bird Areas (IBA). Diversity, distribution including microhabitat preferences, and conservation status of its anurans, being biological indicators of disturbance, were determined. The area was assessed using visual encounter survey and pitfall trapping from October 21 to November 22, 2006 in three elevation gradients (670-1,050masl) with corresponding vegetation types: mixed lowland dipterocarp (1), mixed primary-secondary (2), and upper montane-mossy forest (3). Diet and endoparasite of the endemic *Rana grandocula* and non-endemic *Limnonectes magnus* were noted through digestive tract examination of voucher specimens.

Twenty-one species (four families and 14 genera) were recorded with 12 (57%) endemic and eight (38%) threatened. Highest species richness (R=15), species diversity (H'=2.409), and evenness (E=0.742) in site 2 confirm disturbance and altitude to limit frog species distribution. Microhabitat preferences revealed that majority of the anurans were terrestrial, burrowers, or amphibious species (type IV). Three species were found to be economically important as food. Diet and endoparasite examination of *L. magnus* and *R. grandocula* depict the frogs' high dependence on invertebrate fauna especially of the order Orthoptera, niche overlap, possible competition between endemic and non-endemic species, and the importance of both species as intermediate hosts to cestodes and nematodes. Unsustainable hunting and habitat loss through conversion of forest fragments into abaca and Falcata plantations appear to be the major threats in the area.

Mt. Sambilikan although disturbed, still harbors forest-dependent, endemic, and threatened anuran species. Unfortunately, a high probability of risk exists if present rates of forest destruction and unsustainable community practices continue. A pressing need to create holistic conservation mechanisms, with the involvement of the local community, to effectively protect and possibly connect last fragments of habitats of the local biodiversity, is crucial for the survival of these endemic and threatened species.

Keywords: anuran diversity, environmental health, biodiversity loss, conservation

BSD-53

A PRELIMINARY SURVEY ON THE AVIFAUNA AND HERPETOFAUNA OF ANIBONG, JORDAN, MT. PANGASUGAN RANGE, LEYTE

Karyl Marie B. Fabricante[1], Angeli A. Valera[1], Blas R. Tabaranza, Jr.[2], Dennis A. Warguez[1], Alicia Catalina E. Tabaranza and Michael Edrial[2]

[1]Department of Biological Sciences, College of Science and Mathematics
Mindanao State University-Iligan Institute of Technology
Andres Bonifacio Avenue, Iligan City
Tel.: (063)221-4050 Loc 137; E-mail: karyl_marie@yahoo.com,
angeli_valera@yahoo.com and denwarguez@yahoo.com)
[2]Haribon Foundation for the Conservation of Natural Resources
4F Fil-Garcia Tower, 140 Kalayaan Ave.Diliman,
Quezon City 1101 Philippines

Avifaunal and herpetofaunal species diversity and distribution in Anibong, Jordan, Mt. Pangasugan range were determined. Eight study sites with different habitat types were chosen and assessed. Sampling was done on April 21-May 1, 2006 using visual encounter method, pitfall trapping for herps, mist-netting and Mackinnon's species listing for birds. Fifty-one avifaunal species belonging to 27 families and 44 genera were recorded, of which, 44 species (86%) were found to be endemic. Seventeen herpetofaunal species belonging to six families (Ranidae, Rhacophoridae, Agamidae, Scincidae, Colubridae, Viperidae) were identified with eight (47%) endemics. Four threatened species were recorded. One Anuran, *Limnonectes magnus* (near-threatened), and three bird species namely, *Penelopides panini* (endangered species), *Buceros hydrocorax* (near threatened) and *Coracina mindanensis* (vulnerable). This shows that though the area is generally slightly disturbed, it still serves as an important habitat to endemic and threatened species which are very vulnerable to environmental stressors. Habitat loss as forest fragments are converted into Abaca plantations, Illegal and unsustainable harvesting of resources and wildlife trade stand as grave threats to the local diversity. It is imperative that a more thorough study be conducted to assess sustainable conservation measures that may be applicable for the area, considering that it may be one of the few remaining natural habitats of these endemic species.

Keywords: avifauna, herpetofauna, diversity, environmental threats, biodiversity loss

BSD-55

AN IMPROVED EXON-INTRON RECOGNITION VIA A COMMITTEE OF MACHINES

Jaderick P. Pabico[1], Elmer-Rico E. Mojica[2,3] and Jose Rene L. Micor[2*]

[1]Institute of Computer Science and
[2]Institute of Chemistry, College of Arts and Sciences
University of the Philippines Los Baños, College 4031, Laguna
[3]Department of Chemistry, State University of New York,
Buffalo, NY 14260-3000
Email: [1]jppabico@uplb.edu.ph, [2]elmericomojica@yahoo.com,
[2*]jrlmicor@yahoo.com

The human genome consists of a sequence of gene base pairs that generate proteins called exons. Exons are bounded by subsequences, called introns, that are spliced out prior to translation. In RNA splicing, the current procedure followed by researchers to recognize the gene boundaries is the GU-AG heuristic which has the following motif: exon/GU-intron-AG/exon. However, this motif occurs so frequently that a typical intron will contain several GUs and AGs within it, resulting in many false boundaries being recognized. Several methodologies to automate the recognition of these sites have been employed by other researchers, such as support vector machines, hidden Markov models, and artificial neural networks (ANN), where the reported maximum recognition accuracy on a production set is only 81%. A production set is a set of DNA sequences whose intron-exon boundaries are known but where not used in the development of the model. A committee of machines is a computational methodology where the output of multiple models are combined into a single output. The member models' output are combined using several methodologies such as averaging, boosting, bagging and simple majority voting. It has been shown, both theoretically and empirically, that the output of the committee machine is superior to those of its constituent member models. In this effort, we developed a committee of neural network classifiers trained to classify whether a given 60bp long DNA sequence is an intron-exon (IE) boundary (acceptor site), an exon-intron (EI) boundary (donor site), or not (N). Using the same production set used by other researchers, our committee machine was able to recognize 84% of the DNA sequences, improving the recognition rate by 3%.

Keywords: intron, exon, committee machines, machine recognition

BSD-56

Mitochondrial DNA typing of Human Bone Samples for Forensic Applications

Gayvelline C. Calacal*, Jazelyn M. Salvador, Kristina A. Tabbada, Sheila A. E. L. Franco and Dr. Maria Corazon A. De Ungria

DNA Analysis Laboratory,
Natural Sciences Research Institute, Miranda Hall
University of the Philippines Diliman, Quezon City 1101
Telefax: (63 2) 925-2965; *E-mail: gcalacal@gmail.com, updnalab@gmail.com

Mitochondrial DNA (mtDNA) is characterized by low molecular weight and high copy number, making mtDNA suitable for typing biological samples in forensic cases where evidence has been exposed to harsh environmental conditions for prolonged periods. MtDNA sequencing has therefore been used to analyzed highly degraded remains e.g. disaster victim identification and may yield additional information when conventional DNA testing techniques, such as autosomal Short Tandem Repeat (aSTR) typing, fail to produce informative results. However, the success of different typing techniques performed on human remains varies as a result of the condition of the samples, and therefore procedures for mtDNA typing of degraded human remains should be validated prior to use in Philippine casework.

Human bone samples (n=4) exhumed three to six months after burial were processed using validated DNA extraction procedures. DNA was typed at aSTR markers and sequenced at the mtDNA Hypervariable Regions (HVR) I and II. Nuclear DNA was amplified using the COFiler® multiplex system. PCR amplification of mtDNA was likewise tested with combinations of primers designed to produce amplicons 1) covering the *entirety* of Hypervariable Regions (HVR) I and II (1000, 800 and 400 bp in length) and 2) short stretches (approximately 200 bp in length) *within* the HVR I and II. Amplicons were sequenced using Big Dye Terminator technology.

Analysis showed that while samples buried up to six months yielded aSTR profiles, the results showed allelic and locus dropout, which could lead to loss of information and a reduction in the discriminating power of DNA testing. MtDNA typing results showed that PCR products as long as 800bp could be amplified from bones buried up to six months.

Keywords: DNA typing, identification, mtDNA sequencing

BSD-57

ACUTE AND REPEATED SEPARATIONS ALTER THE DENSITY OF ASTROCYTES IN THE PREFRONTAL CORTEX

R. Antemano[1,2], C. Helmeke[1] and K. Braun[1]

[1]Dept. Zoology/Developmental Neurobiology
Otto von Guericke University, 39008 Magdeburg, Germany
[2]Adventist University of the Philippines
Puting Kahoy, Silang, 1099 Cavite, Philippines
E-mail: r_antemano@yahoo.com

Astrocytes, once considered as merely supporting cells in the brain by only assisting neuronal functions are now implicated to play crucial roles in neuronal migration, establishment and maturation of synaptic contacts during the early development. Relatively, only few reports have shown the influence of neonatal environment on glial plasticity in the medial prefrontal cortex (mPFC), the region that processes, integrates and evaluates memories of learning and experiences. Thus, the impact of separation from the family was investigated on astrocytes of *Octodon degus* pups during the first three postnatal weeks, the critical period for synaptic plasticity in rodents. The expressions of two astrocyte proteins, S100ß and GFAP (glial fibrillary acidic protein) were used to determine the impact between: control, n=5 (CON): undisturbed in the home cage from postnatal day (PND) 1-21; acute separation (AS), n=6: 6-hr separation on PND 21; and repeated separation, n=6 (RS): 1 hr/day separation from PND 1-21. The density of S100ß and GFAP-positive astrocytes was quantified in the subregions of the mPFC namely: anterior cingulate (ACd), precentral medial (PrCm), prelimbic (PL) and infralimbic (IL) cortices. The somatosensory cortex (SSC) was used as a nonlimbic control region. Both acute and repeated neonatal separations altered the density of S100ß and GFAP-positive astrocytes in the mPFC showing increases in S100ß-positive astrocytes in a region and layer-specific manner but decreases in GFAP-positive counterparts. In the SSC, acute separation did not affect the S100ß-positive astrocytes but increased the GFAP-positive counterparts. Taking these findings together, the separation-induced alterations may have consequences in neuron-glia interaction thereby affecting the participation of astrocytes in modulating the synaptic plasticity particularly during the early period of postnatal development. These findings also provide evidence of uniqueness in spatial and temporal specificity of glial response towards a particular environmental stimulation.

Keywords: astrocyte, glia, GFAP, medial prefrontal cortex, S100B, separations

BSD-58

COMPARATIVE STUDY OF THE UTILITY OF ABO BLOOD-TYPING VS DNA ANALYSIS FOR PATERNITY TESTING

Miriam Ruth M. Dalet*, Minerva S. Sagum, Gayvelline C. Calacal, Jazelyn M. Salvador, Kristina A. Tabbada, Lilian P. Villamor, Shiela Ann Edith L. Franco, Sheena Marie B. Maiquilla and Maria Corazon A. De Ungria.

DNA Analysis Laboratory,
Natural Sciences Research Institute, Miranda Hall
University of the Philippines Diliman, Quezon City 1101
Telefax (632) 925-2965; *Email: mrbmdalet@gmail.com, updnalab@gmail.com

DNA typing is the most accurate method in evaluating disputed parentage issues. However, it has been argued that given the current high cost of DNA-based paternity testing, conventional ABO blood-typing should be utilized as an initial screen prior to DNA-based testing. It is thus important to determine the effectiveness of ABO blood typing in determining paternity in comparison with a DNA-based paternity test.

A study of 53 paternity trios was conducted by the UP-NSRI DNA Analysis Laboratory from 2006 - March 2008. Each trio was subjected to DNA testing using 15 Short Tandem Repeat DNA markers and to conventional ABO blood typing. Paternity evaluations using the two methods were then compared.

Only 3 out of 53 alleged fathers (5.66%) could be excluded as being the biological father of the child using ABO blood typing. The remaining 50 alleged fathers could not be excluded as the biological father of the child.

Interestingly, while all 3 ABO paternity exclusions were also excluded using DNA testing, 12 of the alleged fathers that could not be excluded based on ABO testing were excluded by a minimum of 6 STR markers using DNA testing. The remaining 38 alleged fathers (71.69%) were included as the biological father of the child with probabilities of paternity ranging from 99.96% to as high as 99.999999%.

Given these figures, the usefulness of conventional ABO blood-typing for paternity testing is limited and is no longer cost-effective. We recommend the use of autosomal STR DNA typing for evaluations of disputed parentage cases in the Philippines.

Keywords: paternity testing, DNA, ABO blood-typing, probability of paternity, STR markers, paternity exclusion, paternity inclusion

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A SACCHARINE SUBSTRATE-PREFERRING ZYMOMONAS STRAIN AND ITS GENETIC FINGERPRINTING USING ITS-PCR AND RAPD-PCR

Franciso B. Elegado, Daryl Ann Stefanie G. Torralba and Irene G. Pajares

BIOTECH, U.P. Los Baños, College, Laguna

A local Zymomonas isolate (PNCM 1832) was found to preferably utilize brown sugar and molasses than glucose. It was subjected to preliminary DNA fingerprinting tests in comparison with two type culture strains of Zymomonas mobilis (1003 and 1004) and two other putative Zymomonas mobilis isolates (PNCM 1229 and 1231). Intergenic 16S-23S Transcribed Spacer (ITS) forward and reverse primers and five Random Amplified Polymorphic DNA (RAPD) arbitrary primers were used in Polymerase Chain Reaction (PCR) assays. Results of ITS-PCR experiment showed perfectly similar bands generated by Z. mobilis 1003 and 1004 with PNCM 1229 and 1231. On the other hand, PNCM 1832 was different by one major band. Composite Winboot analysis and cluster analysis by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) of the RAPD-PCR amplicons generated was done. The consensus phylogenetic tree showed that for most primers used, the strains can be grouped together, suggesting that they are genetically related, i.e. belonging to the same genera. However, in all RAPD-PCR results, PNCM 1832 was the least phylogenetically related which could have some bearing on its increased utilization of saccharine substrates that may be relevant in bio-ethanol production. Preliminary batch fermentation trials by Zymomonas sp. PNCM 1832 using 20% brown sugar as substrate in a 4-L bench top fermentor gave 7.19% (v/v) ethanol at 87% sugar utilization. However, fermentation efficiency was only 51%, suggestive of the production of metabolites other than ethanol.

Keywords: Zymomonas mobilis, winboot, amplicons