

BIOLOGICAL SCIENCES

BS - 01

**PRELIMINARY REPORT OF ANURANS IN TWO
MOUNTAIN ECOSYSTEMS IN BUKIDNON,
MINDANAO, PHILIPPINES**

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A field survey of anurans was conducted in two mountain ecosystems in the province of Bukidnon, Mt. Kiamo, Malaybalay and Mt. Pantaron range, from January to December 2012. Field sampling was performed using a combination of belt transect, opportunistic and random sampling techniques. Field investigations were done across vegetation and elevation gradients from 750-1,500 meters above sea level (masl), which include an agro-ecosystem, montane, and mossy forests. Baseline data on species richness, local distribution, conservation status, and microhabitat preferences of anurans were determined and documented. The study revealed 21 anuran species belonging to five families and 15 genera, 52% of which were endemic in the Philippines. Moreover, four noteworthy Mindanao island endemic species identified, namely: *Ansonia muelleri*, *Leptobrachium lomarudom*, *Megophrys stejneri*, and *Rana grandocula*. Eight species of anurans were common to both mountains. As to the conservation status, the two vulnerable species recorded were *A. muelleri* and *M. stejneri*, whereas *Limnonectes magnus* was categorized as near threatened. A majority of the anurans was observed in the ground microhabitat especially in the leaf litters, fallen and decaying logs, while some species were collected near the bodies of water, such as rivers and streams which are associated with the indigenous Manobo inhabitants. The results of the survey show that the richness of anurans in the two-mountain ecosystem is high and more species are likely to be found. It is recommended that this two-mountain ecosystem be declared as protected area in order to conserve the endemic and threatened species and ecosystem as a whole.

Keywords: species richness, Anurans, microhabitat, endemic, Mindanao

BS - 02

**SPECIES COMPOSITION AND ABUNDANCE
OF SNAILS (GASTROPODA) IN MT. MALAMBO,
DAVAO DISTRICT PHILIPPINES**

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Mt. Malambo is a petit mountain along the highway of Datu Salumay, Marilog, Davao District. It has peak elevation of 1,379 meter above sea level (masl) and is covered with original or native plants in the fragmented montane forest at the base and mossy near the top. Transect survey for snails was conducted on the western slope to provide information on snail species composition. A total of 6 snail species under one family of snail were listed at the total of 82 individuals. The most abundant species was *Cyclophorus presto* (n=30) and the least abundant species was *Leptopoma perlycidum* (n=5). Mt. Malambo is home for 6 species of snails. The effort to conserve the snail species will also conserve the forest and the fireflies therein as they are food for the fireflies and for ecological balance.

Keywords: Species composition, Abundance, Snail, Mt. Malambo, Philippines

BS - 03

INVENTORY OF ASTEROIDEA SPECIES IN JASAAN, MISAMIS ORIENTAL, NORTHERN MINDANAO

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This study was conducted to assess the sea star population in three selected barangays of Jasaan, Misamis Oriental. The specific objectives of this study were: 1) to collect and identify the Asteroidea species present; 2) to determine the species abundance and diversity; and 3) to determine some physico-chemical parameters in the study stations. Three study stations located in Solana, Bobontugan and San Antonio were established, each with a 100m² plot divided into 100 quadrats about 35-45 m from the shore. Asteroidean species present were counted, collected, and preserved for identification and classification. Sampling was done twice. Photographs of the sea stars and study sites were taken. Physico-chemical conditions during the time of sampling were recorded. Species importance value was computed based on the relative density and relative frequency of the species. There were four species of Asteroidea collected with a total of 74 individuals: *Acanthaster planci*, *Linckia laevigata*, *Culcita novae-guinea*, and *Nardoa tuberculata*. In Solana, the most dominant and the most frequently observed was *N. tuberculata* (IV=1.270, f=0.075); in Bobontugan and San Antonio, the dominant species was *L. laevigata* (IV=0.951, f=0.075 and IV=1.378, f=0.065, respectively). Among the three stations, the most diverse was Bobontugan (H=0.458). The physico-chemical conditions of the three areas did not vary much. pH was 8 and the water was clear, with a slight range of water temperature (27-30°C). The substrate of the three areas was almost the same: coralline, rocky and sandy. Similar associated organisms were found in three areas, except in San Antonio which had tall and abundant *Sargassum*. Based on the Shannon-Wiener diversity classification, species diversity in the three study areas was relatively low with a mean value of 0.402.

Keywords: inventory, Asteroidea species, abundance, diversity, Jasaan, Misamis Oriental

BS - 04

**MACROINVERTEBRATES AS WATER QUALITY
BIOINDICATORS OF SEBASI RIVER
IN OZAMIZ CITY, PHILIPPINES**

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Sebasi River is the water source for domestic, commercial and agricultural needs of several barangays in Ozamiz City. Due to climate change and continuous human related activities, water quality and aquatic organisms are threatened. This descriptive research was conducted to determine the status of upstream, midstream and downstream of the river using macroinvertebrates which are useful biological indicators of change in the aquatic ecosystems. Physico-chemical parameters (air and water temperatures and pH) were measured using standard field methods. Dip and kick-net methods were used to sample macroinvertebrates. Organisms were categorized depending upon their tolerance to organic pollution (sensitive, somewhat sensitive, and tolerant). These were then subjected to biotic index. Results showed that air and water temperatures and pH values were still within the tolerable range. Based on macroinvertebrates, the upstream had total index value of 29 that signified excellent water quality, midstream with total index value of 15 (fair), and downstream with total index value of 8 (poor). This implied that the cleanest water can be found at the upstream while the most impure water is found in the downstream. An environmental protection and proper management of natural resources program in Sebasi River should be implemented.

Keywords: bioindicators, macroinvertebrates, water quality, river

BS - 05

**PHYTOPLANKTON, MACROINVERTEBRATES
AND RIPARIAN, CHANNEL, AND ENVIRONMENTAL
(RCE) INVENTORY OF THE FRESHWATER SYSTEMS
WITHIN PLATINUM GROUP METALS CORPORATION
(PGMC), CLAVER, SURIGAO DEL NORTE**

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The Platinum Group Metals Corporation (PGMC) is one of the many nickel mines in Claver, Surigao del Norte. Two freshwater biological studies had been conducted within its vicinity in the last 10 years: a baseline study in 2002 and a biological assessment in 2010. The present study was performed as a follow-up assessment of the status of the freshwater systems in the mining site by making an inventory of the plankton and macroinvertebrates while performing a riparian, channel and environmental evaluation. This study showed the absence of phytoplankton, the appearance of pollution-tolerant hemipterans and chironomids and confirmed the paucity in macroinvertebrate species reported in 2002. All five freshwater stations within the mine site obtained poor ratings using the riparian, channel, and environmental evaluation compared to the 2010 study where at least 1 station was given a fair rating. Immediate remedial action is recommended to enable natural systems to reverse the degradation of the freshwaters.

Keywords: freshwater biology, mining, pollution, phytoplankton, macroinvertebrates

BS - 06

**ORCHID FLORA OF MT. APO AND MT. KITANGLAD
LONG-TERM ECOLOGICAL SITES
IN MINDANAO ISLAND, PHILIPPINES**

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Orchids are among the most attractive and highly priced plants harvested in the mountains of Mindanao. Unfortunately, several species are already endangered and threatened due to habitat destruction, over-collection and illegal trade. Taxonomic studies are therefore badly needed to identify and assess the status of this economically important flora. Floristic studies were conducted in one-hectare permanent plot established in Mt. Kitanglad, Lantapan and Mt. Apo, Kidapawan. Fieldwork inventory of orchid flora were conducted for *ex-situ* propagation and conservation. Field collections and documentation were made for taxonomic verification of the plant materials. Results gave a total of 15 species of orchids belonging to 11 genera. Species richness was higher in Mt. Kitanglad with 13 species, while Mt. Apo was represented with 10 species. Eight species were found to be common in the two mountain ecosystems. *Mycaranthes* and *Cryptostylis* species were found only in Mt. Apo while *Cystorchis*, *Stichorkis*, *Hippeophyllum*, and *Pinalia* species were unique to Mt. Kitanglad. *Dendrochilum*, *Agrostophyllum*, and *Crepidium* were the most dominant in both sites. On-going *ex-situ* conservation of dwindling orchid populations was initiated for more effective conservation.

Keywords: species richness, orchids, *ex-situ* conservation, long-term ecological sites, Mindanao

BS - 07

FOUR NEW COMBINATIONS FROM THE PHILIPPINE ENDEMIC *Canthium* Lam. (RUBIACEAE): EVIDENCES FROM NUCLEAR AND PLASTID DNA SEQUENCE DATA

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Canthium Lam. (Vanguerieae, Rubiaceae) forms a polyphyletic assemblage based on current phylogenetic studies. The genus is presently delimited to include species with spines. In contrast, several Philippine endemic species of *Canthium*, such as *C. gynochtodes*, *C. oblongifolium*, *C. obovatifolium*, and *C. oligophlebium* do not possess spines. This raises a question on the true generic identities of these *Canthium* species. In this first molecular study of Philippine *Canthium*, the plastid (*trnL-F* region) and nuclear (ITS region) DNA were sequenced and assembled together with the previously published sequences of Vanguerieae to determine its phylogenetic position and true generic affiliations. The majority-rule consensus tree of the Bayesian inference showed a well-supported clade of the whole Vanguerieae. Interestingly, *Canthium oligophlebium*, *C. obovatifolium*, and *C. oblongifolium* were nested within the *Pyrostria* clade (PP=1.00), while *C. gynochtodes* was in *Psydrax* (PP=0.76). These molecular results strongly corroborate with the morphology of *Pyrostria* and *Psydrax*, leading to the establishment of two additional genera in the Philippine's biodiversity. Four novel combinations from the Philippine *Canthium* are here proposed.

Keywords: *Canthium*, ITS (nrDNA), Philippine endemic, *Pyrostria*, *Psydrax*, *trnL-F* (cpDNA)

BS - 08

**MOLECULAR AUTHENTICATION OF SPERMACOCEAE
S.L. (RUBIACEAE) SPECIES FROM WESTERN PANAY
PENINSULA USING MULTI-LOCUS DNA
BARCODES REVEAL A NEW PHILIPPINE ENDEMIC
SPECIES OF *Hedyotis* L.**

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The introduction of DNA barcoding as a technology to discriminate land plants has been restrained by the lack of consensus as to what genetic markers will be used to serve as universal standards in facilitating species identification. Throughout its taxonomic history, the circumscription of the primarily herbaceous tribe Spermacoceae *sensu lato* of the family Rubiaceae has been the subject of disputes among plant systematists due to its anatomical and morphological heterogeneity, leading to problematic authentication of its taxa. In this paper, we used two non-coding (*rps16* intron, *trnL-F* intergenic spacer) loci of the chloroplast DNA to test the possibility of their use as barcodes to validate Spermacocean species of the Western Panay Peninsula. Ten accessions were sequenced from five collected samples and were included in a data matrix comprised of 78 and 81 Spermacoceae GenBank accessions for *rps16* and *trnL-F*, respectively. Single locus resolution ranged from 0-11.48% (*rps16*) to 0-17.68% (*trnL-F*), while the combined loci revealed reasonable success rate of 35.13%. In addition, comparison of the sampled dioecious *Hedyotis panayensis* to extant type specimens supported our proposal for a novel Philippine endemic.

Keywords: biodiversity, DNA barcoding, cpDNA, flora, Spermacoceae

BS - 09

**MOLECULAR PHYLOGENY OF PHILIPPINE *Blechnum* L.
(BLECHNACEAE) BASED FROM *trnL-trnF* (cpDNA)
SEQUENCE DATA**

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The genus *Blechnum* L. (Blechnaceae) comprises 20 taxa (17 species and 3 subspecies) of the Malesian region, 8 of which can be found in the Philippines. Recent molecular studies involving the genus have failed to include Philippine representatives. In this first molecular phylogenetic study on Philippine (PH) *Blechnum*, the *trnL-trnF* (cpDNA) regions were sequenced and analyzed together with the previously published related sequences from the GenBank. Specifically, the present study intended to reassess the monophyly of the genus *Blechnum* and the phylogenetic relationships of the PH *Blechnum* with New Zealand (NZ) and other overseas Blechnaceae species. Two-hundred ninety out of 983 aligned nucleotide positions (29.50%) were found to be parsimony-informative characters. The strict consensus tree revealed a paraphyletic *Blechnum* as other genera (*Doodia*, *Sadleria*, and *Stenochlaena*) are nested within the genus. Interestingly, the PH *B. egregium* is more closely related to the South American *B. brasiliense*. These two *Blechnum* species formed a subgroup together with all the sampled *Doodia* species. Similarly, the two sampled *B. orientale* formed a subgroup with *Sadleria* and *Stenochlaena*. The rest of the PH *Blechnum* species have high affinities with the NZ and Australian species.

Keywords: Blechnaceae, Philippine *Blechnum*, molecular phylogeny, *trnL-trnF*

BS - 10

**COMPARATIVE DIVERSITY OF GROUND-DWELLING
ANTS (HYMENOPTERA: FORMICIDAE) IN TWO
PERMANENT PLOTS IN MAKILING FOREST RESERVE,
LOS BAÑOS, LAGUNA, PHILIPPINES**

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The study of comparative diversity of ground-dwelling ants done in Mt. Makiling Forest Reserve aimed to assess and compare the diversity of PFLA 1 and 3. Data collection was conducted through pitfall trapping and active searching of ants. There was a total of 14 species of ants collected in PFLA 1 and 3. Four species were identified as follows: *Anoplolepis gracilipes* (Formicinae: Plagiolepidini); *Diacamma* sp. (Ponerinae); *Odontomachus infandus*, and *Odontomachus* sp. (Ponerinae). Other species remained unidentified. Results showed that *Odontomachus infandus* was the most dominant species in PFLA 1 while Unidentified Species 2 was the most dominant species collected in PFLA 3. After computing the different diversity measurements, PFLA 1 was found to be more diverse than PFLA 3. The *t*-test showed that PFLA 1 and 3 had significantly different species diversity. It is recommended that more studies about ant diversity be conducted which will further contribute to biodiversity studies.

Keywords: *Anoplolepis gracilipes*, *Diacamma* sp., ground-dwelling ants, *Odontomachus infandus*, *Odontomachus* sp.

BS - 11

**DIVERSITY OF ODONATA: THE LOCAL WAY OF
ASSESSING WATER QUALITY IN LAKE PINAMALLOY,
DON CARLOS AND DOLOGON SPRING,
MINDANAO, PHILIPPINES**

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Water quality assessment of Lake Pinamaloy and Dologon spring, Bukidnon was conducted using diversity indices of Odonata species to provide information as to whether species composition, diversity and status of Odonata in the vicinity of Lake Pinamaloy and Dologon Spring can be used by locals in assessing water quality. A total of 3 families, 11 genera, and 18 species of Odonata are found in Lake Pinamaloy, and 3 families, 13 genera, and 20 species are found in Dologon Spring. The species diversity in Odonata in Lake Pinamaloy and Dologon Spring are low: $H'0.66$ and $H'0.875$, respectively. Field biotic index (FBI) showed medium water quality for both (50.7 and 60, respectively). Species richness of Odonata was higher in Dologon spring with forest fragments and less human activities near the source. Two endemic species were listed and Odoanata are unevenly distributed in the two sites. Distribution varied in two sites with different vegetation types, degree of light penetration, presence of microhabitats and quality water. The results suggest that Odonata can be useful in assessing and monitoring water quality in the absence of ecological kits. The local communities can be empowered to creating water conservation culture, especially when they recognize that Odonata are sensitive indicators for testing water quality.

Keywords: Odonata; diversity; water quality; Mindanao, Philippines

BS - 12

DIVERSITY OF CICADAS IN THREE SELECTED MOUNTAINS OF MINDANAO, PHILIPPINES

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Diversity of cicadas in three selected mountains of Mindanao namely: Mt. Musuan, Mt. Kitanglad and Mt. Hamiguitan. Cicadas are considered for study being biomass converters and indicator of a forest quality. They are dependent on trees for shelter. Hence its diversity indicates habitat quality. This paper aimed to provide information on cicada species composition and species level-diversity in three selected mountains of Mindanao. Data generated may be used for species monitoring, distribution, biogeography and conservation of cicadas. Belt transect, time constraint, transect walk sampling, light trap, malaise trap and pan traps were employed from April 2011 to May 2012. We captured a total of 21 species of cicadas: 8 from Mt. Musuan, 9 from Mt. Kitanglad and 17 from Mt. Hamiguitan. Species diversity level using Shannon-Weiner index showed low level in all sites. In Mt. Musuan $H'0.697$, Mt. Kitanglad $H'0.965$ and Mt. Hamiguitan $H'1.063$. Highest species richness was observed in Mt. Hamiguitan, lowest in Mt. Musuan. Bray-Curtis analysis on species composition showed that all sites are unique habitat for cicadas. This result suggests that cicada species has specificity for habitat. The locals utilize cicadas for food, bait for fishing and for forecasting weather conditions. They also recognized that cicadas are worthy for conservation as they indicate forest quality.

Keywords: diversity, cicadas, mountain, Mindanao, species composition

BS - 13

**LEAF METHANOLIC EXTRACT OF *Ardisia* sp. INHIBITS
ANGIOGENESIS IN THE DUCK *Anas platyrhynchos*
CHORIOALLONTOIC MEMBRANE**

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The present study was conducted to evaluate whether the methanolic extract of *Ardisia* sp. can effectively inhibit angiogenesis in the duck chorioallontoic membrane. Different concentrations of *Ardisia* sp. (2, 4, and 6 mg/ml) were topically applied in the duck chorioallantoic membrane. Histochemical analysis of CAM and histological analysis of the heart were conducted. There was a significant difference between the treatments and the control group, but there were no significant differences of the mean vascular densities between doses. Histochemical analysis of the chorioallantoic membranes using alcian-blue stain showed that the intensity of the stain is less in the treated samples. There were no abnormalities observed in the gross morphology of the duck embryos and in the histology of the hearts. This work showed that the methanolic extract of *Ardisia* sp. has potential anti-angiogenic property.

Keywords: *Ardisia* sp, anti-angiogenic, *Ardisia* leaf methanolic extract, chorioallontoic membrane assay, angiogenesis

BS - 14

BIOLOGY AND ECOLOGY OF THE LAKE TAAL SEA SNAKE, *Hydrophis semperi*

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We studied the basic biology and ecology of the endemic Lake Taal Sea Snake (*Hydrophis semperi*) in Lake Taal, Philippines. Despite its conservation status (vulnerable), it is scientifically understudied. This study provides baseline scientific information on the unique sea snake species occurring in freshwater habitat. Gill net trapping was primarily employed during the sampling period (June-November 2012). Morphometric data were gathered from snake samples with live snakes tagged and released, and dead samples extracted of its gut content. Environmental parameters (*e.g.*, water temperature, light intensity, conductivity) were also collected for multivariate analysis. Out of 112 snakes, mostly collected from the south basin, only 24 individuals belonged to *Hydrophis semperi*. No sea snake was caught in the north basin of the lake. The remaining samples (n=88) were identified as the Little File Snake (*Acrochordus granulatus*) with one recapture from tagged samples (n=33). Snake captures reflect the relatively lower abundance of *H. semperi* and the high capturability of *A. granulatus*. Identification of snake gut contents reveal that *H. semperi* feeds on three families of fish (Gobiidae, Hemiramphidae, and Anguillidae). The sympatric *A. granulatus* were found to contain prey items belonging to only one family (Gobiidae). Prey items found in the gut of the endemic sea snake species suggest that it is a gape-limited generalist predator. *In-situ* observations reveal that *H. semperi* exhibits a “surface-arch” movement distinct from *A. granulatus* when surfacing from underwater. Further observations suggest that both species are strongly associated with rocks and crevices. Higher conductivity values present at the mouth of the river near the lake reflect the marine origins of the lake and its volcanic nature which may have created the conditions which are suitable for the survival of *H. semperi*. This study provided insights for further investigation.

Keywords: hydrophiinae, hydrophis, Lake Taal, freshwater, sea snakes

BS - 15

**EVALUATION OF THE GLYCEMIC EFFECT OF
Parameria laevigata (A. Juss) Moldenke IN NORMAL
AND ALLOXAN-INDUCED DIABETIC JUVENILE
MICE (*Mus musculus* L.)**

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The rise in the incidence of diabetes has been alarmingly rapid in both developed and developing countries. In developing countries medicinal plants have always played a significant role in the maintenance of health and management of diseases. In the Philippines, there are many plants with reported antidiabetic property but are not scientifically tested yet. Using male mice as model organism, the ethanolic extract from the leaves of *Parameria laevigata* was tested for hypoglycemic activity in both normoglycemic and alloxan-induced diabetic mice. The extract reduced blood glucose levels (BGL) in a dose-dependent manner. When given to normal and alloxan-diabetic mice, the extract at 100 mg/kg bw reduced the level of hyperglycaemia by 20% and 45%, respectively, 1 hr after treatment compared to controls. Moreover, a significant reduction in BGL was noted in diabetic mice 2 and 3 hrs post treatment ($P < 0.05$) at a dose of 50 mg/kg bw. These results clearly indicate that the ethanolic extract from the leaves of *P. laevigata* has high antidiabetic potential in a dose-dependent manner. Further characterization of the active components of these plants is warranted to understand the mechanism of its hypoglycemic action.

Keywords: alloxan, antidiabetic, hypoglycemic, normoglycemic, *Parameria laevigata*

BS - 16

SURVIVAL RATE OF FEMALE WHITE MICE *Mus musculus domesticus* TREATED AT VARIOUS MONOSODIUM GLUTAMATE CONCENTRATIONS

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A completely randomized design (CRD) experimental set-up was conducted to determine the survival rate of *Mus musculus domesticus* at various concentrations of monosodium glutamate (MSG). Adult female white mice from 20 inbred strains were used as test organisms. Four different treatment groups of free-access oral drinking solutions at various MSG concentrations were prepared, namely: $T_1=74\%$, $T_2=55\%$, $T_3=37\%$, $T_4=18\%$; T_5 was the control group that contained only distilled water. In each group, there were four replicates that were caged individually. Each mouse received normal food diet and 20 mL of drinking solution daily at 7:30 AM for 30 days. The volume of the solution consumed by each mouse 24 hours later was then measured. Survival rate of the mice were determined. Statistical analysis was carried out using Analysis of Variance (ANOVA) to determine if there were significant differences in the mean intake for the 30-day period of observation, between and among the experimental groups and the control group. To determine which groups differ, Tukey HSD was utilized for multiple comparisons. Results showed that the F value of 31.300 was highly significant at $\alpha=0.01$; that is, at 99% confidence that there were significant differences between the groups. From the results there were no significant differences between the mean MSG intake among the four treatment groups; however, the mean MSG intake of the four treatment groups differed significantly from the control group. This implied that the higher the concentration of MSG solution consumed, the lower the survival rate, as given by a correlation value of -0.5777 , or equivalently, a determining factor of $(-0.5777)^2 \times 100\% = 33.29\%$. This means that the concentration of MSG solution significantly affects survival rate of the white mice.

Keywords: monosodium glutamate, oral drinking solutions, oral intake, white mice, treatment, mortality, survival rate

BS - 17

**DNA FINGERPRINTING AND GENETIC DIVERSITY
ANALYSIS OF PHILIPPINE SABA (*Musa balbisiana* Colla)
CULTIVARS USING MICROSATELLITE MARKERS**

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Saba (*Musa balbisiana* Colla) is an endemic crop and one of the most important cultivars of banana grown in the Philippines. In recognition of the importance of Philippine Saba as a source of food, it is essential to identify Saba cultivars with good fruit quality and high potential for processed food and industrial application. Microsatellites markers were used to generate DNA fingerprints and to characterize the genetic diversity among 14 Philippine Saba cultivars. A total of 45 primer pairs obtained from INIBAP (International Network for the Improvement of Banana and Plantain) were tested for PCR amplification and polymorphism across the 14 Saba cultivars. Of these primers, 19 were polymorphic markers and two were monomorphic markers. A total of 67 alleles were generated, with a mean of 3.5 alleles per locus, ranging from 2 to 8 alleles. The resolving power of molecular markers measured as the Polymorphism Information Content (PIC) ranged from 0.05 to 0.94. The dendrogram using UPGMA-SAHN cluster analysis based on microsatellite amplification and polymorphism showed that the Saba cultivars clustered into ten groups at the 88 % similarity level. Cluster analysis separated the cultivars of *Musa balbisiana* Colla from genotypes of *Musa acuminata* Colla. The 19 polymorphic SSR primers were shown to be able to identify and differentiate the 14 Saba cultivars. The results of this study provide useful information for proper identification of Saba cultivars suitable for specific needs of the industry.

Keywords: Saba, *Musa balbisiana*, microsatellites, polymorphism, genetic diversity, DNA fingerprinting

BS - 18

**GENETIC DIVERSITY ANALYSIS OF PILI
(*Canarium ovatum* Engl.) USING CROSS-SPECIES
AMPLIFICATION OF SIMPLE SEQUENCE REPEATS**

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Pili (*Canarium ovatum* Engl.) has its center of diversity in the Bicol region. Due to a high degree of open pollination, pili trees grown from seeds exhibit variability in many important horticultural characters. This study used Simple Sequence Repeat (SSR) markers from papaya (*Carica papaya*) and Chinese white olive (*Canarium album*) to assess the genetic diversity of 95 pili accessions obtained from the collections of the Crop Science Cluster (CSC): UPLB, DA Pili Research and Technology Center, Tabaco, Albay, and three private pili farms in Barangay San Rafael, Bulusan, Sorsogon. Five SSR primers (SSR12, SSR31, *CasC120*, *CasA131* and *CasC183*) produced high quality, polymorphic PCR products from genomic DNA. Thirty seven alleles were obtained using the five SSR primer pairs with an average of 7.4 alleles per marker. The average Polymorphism Information Content (PIC) of the five primers was 0.7660 which indicated their capability to detect and quantify genetic diversity in *C. ovatum*. Cluster and ordination analyses using NTSYSpc software gave three major clusters at 0.60 similarity coefficient. Group I consisted of mostly IPB-CSC accessions, some accessions from Pili Drive (PD)-CSC and two varieties, Katutubo and Laysa. Group II was mostly PD-CSC genotypes with some Bicol accessions. The other five registered varieties - Lanuza, Magayon, Mayon I, Orolfo and Magnaye - clustered together forming Group III. Cross-species amplification of SSR markers successfully revealed the high genetic variation among the pili genotypes studied.

Keywords: pili, genetic diversity, simple sequence repeats, DNA markers, *Canarium*

BS - 19

COMBINING ABILITY ANALYSIS OF WHITE CORN GENOTYPES FOR NITROGEN USE EFFICIENCY IN IRRIGATED AND MOISTURE-LIMITING CONDITIONS USING THE SAND CULTURE TECHNIQUE

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We report the results of combining ability analysis of a full diallel mating design of eight white corn inbreds. This study was conducted to identify superior genotypes in terms of nitrogen use efficiency under irrigated and moisture-limiting conditions. We evaluated 36 progenies comprising 8 selfs (parentals) and 28 F₁ hybrids for NUE, plant dry matter, plant height, and root volume during the vegetative stage using sand culture technique. We employed three levels of nitrogen (*i.e.*, 0, 60 and 120 kg/ha) and two water treatments (*i.e.*, irrigated and drought-imposed) in a split-split plot in completely randomized design with three replications under greenhouse conditions. We analysed the data on plant dry matter for combining ability using Griffing's method 2 (model 1). Analysis of variance showed that the performance of the genotypes tested for NUE, plant dry matter, plant height, and root volume vary across nitrogen and water treatments. Combining ability analysis revealed that CML377 has the highest General Combining Ability estimate (GCA = 0.0553) among the rest of the parentals, which implies that it would contribute to good plant dry matter production in a wider array of crosses. We noted that CML377 was a common parent for the two F₁ hybrids that perform consistently well in various nitrogen and water treatments. These F₁ hybrids were also among those that attained the highest SCA estimates for all genotypes that were evaluated. The components due to SCA (0.0335) were found to be higher than that of GCA (0.0014), which means that the portion of the genetic effects due to dominance is higher than the additive portion. We recommend CML377 as parental for generating subsequent single-cross combinations in white corn breeding for drought tolerance.

Keywords: white corn, combining ability analysis, nitrogen use efficiency, drought, sand culture technique

BS - 20

MOLECULAR CLONING, IDENTIFICATION AND CHARACTERIZATION OF RICE *DSS* GENE ENCODING A CYTOCHROME-P450 IMPLICATED IN GIBBERELLIN BIOSYNTHESIS

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Gibberellic acid (GA) is a phytohormone that controls many aspects of plant development. In this study, we successfully cloned and characterized rice *DSS* gene encoding a cytochrome-P450 (CYP450) involved in various plant metabolisms. The gene was isolated via map-based cloning from dwarf mutant with small seeds and dark green leaves (*dss*) from *Oryza sativa ssp. japonica* cultivar *Hitomebore* population that had been treated with ethyl-methanesulfonate. The gene locus was mapped in chromosome 3 using simple sequence repeats (SSR) and In-del markers to about 117 kb using the F₂ segregating plants from the cross between mutant and the *Indica* cultivar *Kasalath*. Comparing the delineated sequence of the mutant to a reference sequence, *Hitomebore* found a base change (A→T) which resulted in an amino acid change from glutamic acid to valine. GFP fusion confirmed that it was localized in the endoplasmic reticulum, as observed in other CYP450 gene families involved in mediated stage of GA biosynthesis. Phytohormone assay revealed that the *dss* mutant had a positive response to GA, informatively important for exploring the gibberellins' molecular mechanism using the *dss* mutant. The transformants from RNAi had reduced transcript level and exhibited dwarf phenotype. Accordingly, overexpression of the *DSS* gene strongly confirmed that the gene indeed controls plant architecture in rice.

Keywords: gibberellic acid, phytohormone, cytochrome-P450, map-based cloning, RNAi

BS - 21

**FUNCTIONAL ELUCIDATION OF CONTIG 34 OF
Marsupenaeus japonicus TO WHITE SPOT SYNDROME
VIRUS (WSSV) BY dsRNA INTERFERENCE**

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The genome of the kuruma shrimp, *Marsupenaeus japonicus*, is thus far incomplete, yet it may hold many answers to the immunity response of the shrimp to different pathogens, including White spot syndrome virus (WSSV), one of the most destructive viral diseases among crustaceans, causing one hundred percent (100%) mortality within 3 to 7 days of infection. Contig 34 of *M. japonicus* was found to be homologous to the WSSV genome and might play a role in the infectivity of the virus. To verify this, RNA was extracted from shrimp samples for gene expression, followed by dsRNA synthesis, and interference by injection. Experimental samples were challenged with WSSV, while PBS and GFP were used as controls. Mortality data revealed that contig 34 inferred some protective effect with a survival rate of 32% at Day 5 p.i.

Keywords: host-virus, WSSV, RNAi, kuruma shrimp, contig

BS - 22

**SCREENING OF A GALACTOSE- SPECIFIC LECTIN
GENE IN *Bauhinia purpurea* L. THROUGH PCR ANALYSIS**

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Lectins constitute a class of proteins that bind reversibly to mono- and oligosaccharides. Studies conducted on lectins support the claims of its antiviral, anti-bacterial, anti-fungal and anti-insect activities. Although lectins cannot alter the structure and permeability of the cell membrane and cannot change the normal intercellular activity of bacteria through its attachment to extracellular glycans on bacterial cell walls, lectins can inhibit bacterial activity by blocking their motility. Because lectins are able to recognize and bind to glycoconjugates of animal cell membranes, lectins are used in glycoconjugate isolation, as well as in studying cell structures and blood typing. Their ability to precipitate or agglutinate cells also make them of great use in immunology, cancer research and toxicity studies. In the present study, genomic DNA from the leaves of *Bauhinia purpurea* L., commonly known in the Philippines as butterfly tree, was extracted. Genomic DNA was amplified using lectin gene primers through the Polymerase Chain Reaction. PCR amplification yielded a single band with a molecular weight of 1,636 bp. This result is parallel to the finding of the lectin gene identified in Brazilian *Bauhinia variegata*. It suggests that the *B. purpurea* widely grown in the Philippines may be a source of the lectin protein which is of great interest due to its carbohydrate-binding activity. Sequencing of the PCR products for the lectin gene is underway.

Keywords: lectin, glycoconjugate, *Bauhinia purpurea*, immunology, gene

BS - 23

**TISSUE CULTURE TECHNIQUE FOR CLONAL
PROPAGATION OF NIPA PALM (*Nypa fruticans*
Wurmb., ARECACEAE)**

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In the Philippines, nipa palm is becoming an important source of industrial and many other derivative products. Recently, research on nipa has focused on its potential use as a biofuel crop because it has several advantages compared with other biofuel-alcohol crops. However, making industrial alcohol from nipa is hampered by the availability of superior planting materials in a large quantity. *In vitro* clonal propagation is a promising alternative for producing large quantities of uniform planting materials of high quality. This is the first attempt to develop *in vitro* clonal propagation technique for nipa using embryos from mature fruits of nipa. Sterilization of explants using 5.25% NaClO with 5 drops of Tween20 for 20 minutes gave the best result among the four sterilants tested with 90% decontamination. MY3 (Euwens, 1978), N6 (Chu et al., 1975), and MS (Murashige and Skoog, 1962) basal media with different concentrations of 2,4-D, Ki, and IAA in different combinations were evaluated for shoot induction and root formation. To prevent the tissues from browning, all media were supplemented with 0.25% activated charcoal. MY3 medium supplemented with 7.0 mg/L 2,4-D generated the most number of germinated plantlets. The embryos germinated after three weeks in culture and eventually developed into green plantlets. Plants with 3-4 leaves were transferred into different rooting media. Root formation was observed on MS medium with 5.3 mg/L 2,4-D. Clonal propagation was performed by cutting the plantlets longitudinally along the shoot apical meristem into four sections and cultured in the regeneration medium. The results of this experiment indicated that it is possible to produce 200 seedlings from one seed of nipa in one year at 80% survival rate through *in vitro* clonal propagation, while conventionally it takes at least 5-6 years to generate 15-36 seedlings at 60-93% germination rate.

Keywords: *Nypa fruticans*, nipa palm, *in vitro* clonal propagation, alcohol, biofuels

BS - 24

**UNIQUE DNA FINGERPRINTS IDENTIFY
THE SEVEN NSIC-REGISTERED PILI
(*Canarium ovatum* Engl.) VARIETIES**

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Pili (*Canarium ovatum* Engl.) is a fruit tree species endemic in the Philippines with its center of diversity in the Bicol region. There are seven pili varieties registered with National Seed Industry Council (NSIC)-registered varieties: Katutubo, Laysa, Lanuza, Magnaye, Magayon, Mayon I, and Orolfo. To date, there are no reports on pili variety identification using DNA markers. This study sought to develop a DNA fingerprinting system for the seven NSIC-registered varieties using cross species amplification of simple sequence repeat (SSR) markers from papaya and *C. album*. SSR markers from papaya (SSR7, SSR12, SSR31, SSR34, and SSR38) and *C. album* (*CasA131*, *CasA183*, *CasC120*, *CasC242*, and *CasC254*) amplified a total of 36 polymorphic alleles. The average Polymorphism Information Content (PIC) of the ten SSR markers was 0.61 suggesting their potential for variety differentiation. Cluster analysis using NTSysSpc software of the SSR markers grouped the seven varieties into four at 0.80 coefficient of similarity: Group I– Katutubo and Orolfo; Group II–Magnaye and Magayon; Group III–Lanuza and Mayon I and Group IV–Laysa. Furthermore, the ten SSR markers revealed unique DNA fingerprints for each of the seven pili varieties. We have generated specific DNA fingerprints for the seven NSIC pili varieties that can differentiate them from each other.

Keywords: pili, *Canarium ovatum* Engl., variety identification, simple sequence repeats, fruit morphology

BS - 25

DOUBLE-STRANDED (ds) RNA ISOLATION AND MOLECULAR CHARACTERIZATION FROM THREE WILD-TYPE AND NINE REDUCED GENOME COMPLEMENT STRAINS OF *Rhizoctonia solani* ISOLATES

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The presence of dsRNAs in pathogenic fungi can either increase or decrease their pathogenicity depending on the type and concentration of dsRNA present in them. This study utilized 12 isolates of *Rhizoctonia solani* for isolation and molecular characterization of dsRNA: three wild type strains that are heterokaryons (RS 29, RS 114 and TE2-4) and nine reduced-genome complement strains or mutants (RS 29.5, RS 29.6, RS 29.7, EGR4, EGR7, 123E, T2, TOM7, and strain 115) that are homokaryons. The presence of dsRNAs from four age groups of *R. solani* (25-day old, 50-day old, 75-day old and >75-day old) was determined. The dsRNAs were characterized according to their sensitivity to DNase and RNase and size fractionation based on electrophoretic mobility. All age-group samples showed dsRNA in the homokaryon isolates EGR4 and T2. Also, 50-day old age-group samples showed dsRNA in RS 29. Moreover, >75-day old samples showed dsRNA in RS114 and TE2-4. Observed sizes of dsRNA ranged from 2.1 kbp (T2) to 3.3 kbp (EGR4); concentration of the isolates varied from 0.065 ug/uL to 3.16 ug/uL. Interestingly, dsRNA of TE2-4 was found to be a candidate for specific quantitation using a dual-labeled oligonucleotide probe.

Keywords: *Rhizoctonia solani*, dsRNA, heterokaryon, homokaryon, fungi

BS - 26

**FRUIT COLOR AS AN INDICATOR OF SEED
GERMINATION, SEEDLING PERFORMANCE AND
OIL CONTENT OF *Jatropha curcas* L**

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The escalating prices of imported crude oil by the Philippines prompted the government to develop measures to help the country reduce its use of fossil fuels through greater utilization of indigenous energy resources. One of the species identified as a potential source of biofuel for biodiesel production was *Jatropha curcas* L. This study sought to: (1) document the changes of fruit color from the time the flower emerged until the fruit becomes black; (2) determine the fruit color of jatropha that yields the highest oil content for the production of biodiesel; and (3) assess the performance of jatropha seeds selected based on fruit maturity relative to germination capacity and seedling growth. The experiment was laid out in Randomized Complete Block Design with four treatments and three replications. Changes in fruit color starting from the development of fruit until the color turned black were documented for a period of 34 days using the Royal Horticultural Color Chart (RHCC). Seeds produced during the dry season had higher oil content than seeds produced during the wet season. Seeds taken from yellow fruits had the highest oil yield in both seasons. Seeds taken from black colored fruits during the dry season and dark yellow for the rainy season are recommended for better germination and growth. Fruit maturity was been found to have an influence on the germination capacity, growth performance of jatropha seeds and seedlings, and percentage crude fat content. The best maturity color of jatropha for oil yield production was yellow for both seasons. Black fruits were the most promising in terms of germination and growth performance.

Keywords: *Jatropha curcas*, fruit color, crude fat content, oil yield, seed germination

BS - 27

**ACCUMULATION OF TANNIN IDIOBLASTS AROUND
THE VASCULAR TISSUES OF *Medinilla magnifica*
(MELASTOMATACEAE): IMPLICATIONS FOR
A PLANT'S SYSTEMIC DEFENSE**

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Histological localization of tannin idioblasts in vegetative and reproductive organs of *Medinilla magnifica* was investigated. Histochemical tests confirm that tannin idioblasts are densely accumulated around vascular tissues of almost all structures of the plant. In the vegetative organs, tannin idioblasts conspicuously ensheath major and minor vascular bundles of the leaves and they heavily outline the interior of the vascular cylinder of the stems. The same pattern of accumulation was observed in the reproductive organs where tannins densely surround the vascular tissues of the flower, reaching even up to the funiculus of the ovules and the filament of the anthers. The presence of tannins in plants has long been interpreted as an important systemic defense against pathogens. Hydrolysable tannins have been proven to exhibit significant antimicrobial activity against a number of pathogens, including *Botrytis cinerea*, a necrotrophic fungus that affects many plant species. Various studies have determined that *M. magnifica* contains a considerable amount of hydrolysable and condensed tannins. The distinct pattern of histological localization of tannins in *M. magnifica* suggests advanced systemic defense of the plant against infection which could be a good prospect for future investigations.

Keywords: *Medinilla magnifica*, tannin idioblasts, vascular tissue, hydrolysable tannins, plant's systemic defense

BS - 28

**CHARACTERIZATION OF TWO SPECIES OF *Hylocereus*
Britton and Rose (DRAGON FRUIT) THROUGH
MORPHOANATOMY AND HISTOCHEMISTRY**

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Hylocereus or dragonfruit is an exotic fruit, which is gaining popularity in the Philippines due its high antioxidant content. Its taxonomic classification however is vague. The morphological, anatomical and histochemical properties of *Hylocereus undatus* (white-fleshed) and *H. polyrhizus* Britt. & Rose (red-fleshed) were elucidated. Both species of *Hylocereus* are climbing or hemi-epiphytic cacti with triangular, branching, succulent, green and spinous stems. Their root system is adventitious. The flowers are white in color, night blooming, large, bell-shaped, epigynous, and perfect. Fruits are berry type, with scales and numerous seeds. The two species can be distinguished by several characteristics. *H. undatus* has brown undulate stem margin, less spinous leaves, cream to white flower petaloid color, yellowish green sepaloid color, fruit pulp color is white and not sweet. For *H. polyrhizus*, the stem margin is green and straight, leaves are more spinous, petaloid color is white or yellow, sepaloid color is pinkish-red, purple to violet, fruit pulp color is magenta and sweet. *H. polyrhizus* has more scales (mean = 31) than *H. undatus* (mean = 21). *H. undatus* topmost scale is longer (mean = 5.05 cm) than *H. polyrhizus* (mean = 4.19 cm). In terms of anatomy, the two species are very similar. Transection of the roots and stems show eustelic type of stele, collateral vascular bundle and uniseriate root epidermis. Young aerial roots have pith and more xylem ridges than young terrestrial root in both species. Stem epidermis is multiseriate. Root and stem cortex are parenchymatous and multiseriate with secretory canals. The red dragon fruit (*H. polyrhizus*) generally contains more phytochemicals than the white dragon fruit (*H. undatus*). This includes alkaloids, tannins, saponins, amygdalin, organic acids and fats and oils. It is concluded that the two plants are of the same genus but of different species.

Keywords: *Hylocereus*, dragon fruit, morphology, anatomy, histochemical test

BS - 29

**COMPARATIVE STUDY ON THE FLOWERING PATTERN,
FLOWER AND POLLEN MORPHOLOGY OF *Cassia fistula*
Linn. AND *Cassia alata* Linn. (FABACEAE)**

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Pollen grains of the species of the family Fabaceae are easily transported by wind and may cause many allergies like asthma, allergic rhinitis, and hay fever. This study was conducted to compare the flowering pattern, flower, and pollen morphology of *Cassia fistula* Linn. and *C. alata* Linn. Similarities in qualitative characters in both species in flower and pollen morphological characters were observed. Similarities in floral morphology include the type of flower, floral symmetry, internodal elongation in flower, calyx, corolla, attachment of filament to anther lobe, and length of stamens. They differ in the type of inflorescence which is spike in *C. alata* and pendulous raceme in *C. fistula*, form of corolla in *C. fistula* is rosaceous while caryophyllous in *C. alata*. The attachments of filament to anther lobe are all basifixed. For pollen morphology, the species differ in the shape which is circular in *C. fistula* and triangular in *C. alata*. The flowering pattern of *C. fistula* and *C. alata* showed similarities in the months when flowers are numerous, but they differ in the duration and frequency of flowering. *C. fistula* flowered numerous during March–May and September–November, 2-3 times in a year with the average duration of 35 days, while in *C. alata*, it flowered numerous during March–May and September–December, 3-4 times in a year with an average duration of 38 days.

Keywords: *Cassia fistula*, *Cassia alata*, pollen grains, flowering pattern, flower

BS - 30

FIBER MORPHOLOGY AND RECOVERY, CHEMICAL COMPOSITION AND PULP PROPERTIES OF ABACA (*Musa textilis* Nee) cv. Inosa HARVESTED AT DIFFERENT STAGES OF STALK MATURITY

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The morphological, chemical, physical and pulp properties of abaca fibers cv. Inosa harvested at different stages of stalk maturity was investigated to determine the suitability of the fibers in the production of pulp and paper products and to explore the possibility of getting maximum extractable fibers from abaca leafsheaths using different stripping methods. The fibers were extracted using the mechanical spindle stripping and the modified spindle stripping methods. Abaca cv. Inosa at 8-10 months old (young) already possess the inherent desirable characteristics of fibers for pulp and paper, such as extremely long fiber cells (over 3 mm), thin cell walls, low runkel ratio (less than 0.70), high flexibility coefficient, and high slenderness ratio. The morphological dimensions of fibers taken from young stalks were already comparable to fibers taken from mature stalks (18-24 months old). Fibers from 8-10 months old abaca already possessed the desirable chemical properties for pulping as shown by the characteristic low lignin and ash content, high alpha-cellulose, holocellulose, and hemi-cellulose contents, comparable with those from intermediate and mature stalks. The average pulp recovery, Kappa number, and viscosity of pulp were higher in all the three stages of stalk maturity compared to Laylay and Linawaan cultivars. The maximum fiber yield potential of the abaca cv. Inosa was not yet attained at early stage of maturity. Fiber yield of 8-10 months old abaca stalks was only 21% to 32% of those obtained from mature stalks, but usage wise, the fibers are already suitable for pulp and paper production. Fiber recovery was significantly increased by almost 100% using the modified spindle stripping method resulting in a 64% increase in net income compared to the standard stripping method.

Keywords: fiber morphology, chemical composition, pulp properties, Inosa, stalk maturity

BS - 31

**DEVELOPMENTAL ANATOMY OF OIL CELLS, OIL
GLANDS AND JUICE SACS IN THE FRUIT AND SEED
OF *Citrofortunella microcarpa* Bunge (CALAMANSI)
AND ITS IMPLICATIONS ON THE UTILIZATION OF
CITRUS PEEL AND SEED WASTE**

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There are several reports on the uses of *Citrofortunella microcarpa* (calamansi) juice which varies from medicinal, culinary, and industrial, but few studies have been reported on the uses of the exocarp (fruit peel), juice sacs, and seed. Investigating their uses can convert a waste material into a product. The essential oils present in the rind and seeds are one example. This study on the development of the oil cells, oil glands, and juice sacs of the calamansi fruit and seed at different stages of development was carried out to maximize the extraction of oil for use as fragrance and to minimize peel and seed wastes. Samples were subjected to ethanol-xylol for dehydration and clearing. Nile blue sulfate was used for histochemical tests and image analysis was done to obtain measurements. Results showed that the count of oil glands in a 1 mm² area decreases as the surface area of the fruit increases. As the fruit ages, the oil glands also mature and increase in size, from 1% in young fruits to 45% in mature fruits, which allows them to store more essential oils. Also, an increase in the seed size of the fruit as it ages may imply a higher oil storage capacity in larger seeds due to an increase in the total number of oil cells. The fragrance of the essential oils extracted from the exocarp and juice sacs had the distinct fragrance of limonene although varying in strength. It was also observed that the seed extract had the distinct bitter aroma of limonoids.

Keywords: oil glands, oil cells, juice sacs, essential oils, limonene, limonoids

BS - 32

HERITABILITY OF FACIAL SHAPES AMONG MARANAOS IN LANAOS DEL SUR

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The Maranaos are one of the tri people in the Philippines who engage in intermarriage. This study sought to identify aspects of the shapes of the faces which have high probability of being inherited or transmitted to the offspring of Maranao couples. A total of 240 individuals (from 40 families) from different clans in Lanao del sur took part in the study. Digital images were used and following standard procedures and then analyzed using the method of geometric morphometrics. A total of 39 landmark points were digitized and the X and Y coordinates of these points were used as input for relative warp analyses. Tests for correlation between the relative warp scores of the faces of parents and their offspring were used as measure of heritability. Results showed significant correlation in the shapes of the faces of mother and their sons (P -value = 0.04); in the shapes of the jaws of mothers and her offsprings (Mother-daughter: $3.71E-05$; Mother-son: $4.04E-08$); and the jaw of the father and his daughter (P -value: 0.018). The results of the study are discussed in the light of possible modes of inheritance of the shapes of the face and that of the jaw. Maternal inheritance and the influence of maternal effect genes are explored as possible explanation for the observed correlation in the shapes of the faces of mothers and her progenies.

Keywords: geometric morphometrics, heritability, facial shape, Maranao

BS - 33

OPTIMIZATION OF FERMENTATION MEDIUM OF PHILIPPINE ACTINOMYCETE FOR INSECTICIDAL ASSAY

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Biopesticides have been gaining increased attention and interest among those concerned with developing environmentally friendly and safe integrated insect management. This study sought to evaluate different available and cheaper carbon and nitrogen sources as substitute for production of bioactive compound from Philippine actinomycete for biopesticides. The fermentation medium of actinomycete sp. was optimized to elevate the yield of fraction with insecticidal activity. The effect of independent variables of medium composition, corn starch substitution with molasses, and corn steep liquor with coconut paring meal and whey were investigated. The brine shrimp assay showed that the medium substituted with molasses had the highest number of dead shrimp (94%) after 3 days of fermentation and killed 95% of mosquito larvae after 4 days of fermentation. However, the actinomycete isolate was not effective against corn borer even with the control and substituted medium. Thin layer chromatography showed the separation of bioactive compound in different substituted media. The present study showed the effectivity of Philippine actinomycetes in controlling mosquito larvae using modified media but not effective against corn borer.

Keywords: actinomycetes, mosquito larvae, fermentation medium, agar plug assay

BS - 34

**A NOVEL PHILIPPINE FRESHWATER CYANOBACTERIUM
WITH THERMOSTABLE BROAD SPECTRUM
ANTIBIOTIC ACTIVITY**

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Antibiotic-resistant infections continue to be one of the most dreaded global health threats today. To identify potential new and endemic sources of novel drugs, 20 microalgal isolates from selected Philippine water ecosystems were investigated. The microalgal extracts were screened for antibiotic potential against *Staphylococcus aureus* and *Escherichia coli* using the Kirby-Bauer test. The 16S rDNA of the cyanobacterium from Pagsanjan, Laguna, which showed antibiotic activity was sequenced. The cytosolic fraction was able to inhibit the growth of *S. aureus* (average microbial index of 3.67). If boiled, the cytosolic fraction was also able to inhibit the growth of *E. coli* (average microbial index of 1.92). Preliminary minimum inhibitory concentration of the crude extracts showed strong antimicrobial activity as well as thermostability even after a 10^{-4} dilution and boiling for 5 minutes. Phylogenetic analysis based on the 16s rDNA showed the Pagsanjan isolate to be most closely related to an uncultured gamma proteobacterium (98% maximum identity). While this prokaryote was formerly deemed viable but not culturable, results suggest that the culture techniques used in this study allowed axenic cultivation of the prokaryote under laboratory conditions. This study presents a novel Philippine cyanobacterium isolate with thermostable broad spectrum antibiotic activity. We envision the crude extract, if further purified, to become a potential new drug against antibiotic resistant bacterial pathogens.

Keywords: cyanobacteria, antibiotics, antibiotic resistance, cytosolic fraction, Pagsanjan

BS - 35

**CUTANEOUS BACTERIA OF FROGS COLLECTED FROM
MT. PALAY-PALAY MATAAS NA GULOD NATIONAL PARK,
CAVITE FOR ANTI-CHYTRIDIOMYCOSIS ACTIVITY**

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Chytridiomycosis is a disease in amphibians caused by a chytrid fungus *Batrachochytrium dendrobatidis* and has been implicated as the causal agent of mass mortality and amphibian extinctions. Mitigating strategies are thus among the major concerns in the conservation of amphibian population and biodiversity worldwide. Reports have shown that microorganisms found on the skin of amphibians may combat chytridiomycosis. In this study, cutaneous bacteria from frogs were isolated and examined for anti-chytridiomycosis activity. Eight species of frogs were collected in Mt. Palay Palay Mataas na Gulod National Park in Cavite - a site positive for the presence of chytrid fungus - including *Hylarana similis*, *Limnonectes woodworthi*, *Occidozyga laevis*, *Platymantis mimulus*, *P. corrugatus*, *P. dorsalis*, *Rhacophorus pardalis*, and *Polypedates leucomystax*. Bacteria were isolated from the skin of frogs by swabbing both the ventral and dorsal surfaces of the body and inoculation in R2A agar and incubation at 23±3°C for 24 hours. Chytrid fungus was determined by swabbing the ventral surface of the frog and inoculated in 1% tryptone agar incubated at 23±3°C for 5-7 days. Isolated bacteria were tested for potential inhibitory properties against chytrid fungus. Results showed that some cutaneous bacteria of frogs have potential property to inhibit the growth of chytrid fungus *in vitro*. Skin bacteria of frogs may contribute to their defense and immunity against the lethal chytridiomycosis.

Keywords: cutaneous bacteria, chytrid fungus, *Batrachochytrium dendrobatidis*, chytridiomycosis, frogs

BS - 36

**DIVERSITY OF ENDOGENOUS BANANA STREAK
BADNAVIRUS (BSV) SPECIES IN NATIONAL AND
SOUTHEAST ASIAN MUSA GERMPLASM COLLECTION**

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Banana Streak Virus (BSV) is the most widely distributed and considered potentially the most threatening among the viruses of banana because viral sequences are already integrated in the *Musa* genome and becomes pathogenic (episomal BSV). In determining the frequency and distribution of endogenous BSV sequences across the national and Southeast Asian germplasm collections, the accessions were indexed through standard PCR and multiplex PCR using BSV F1/R2 and species-specific primers, respectively. Results showed the presence of three known BSV species in the collection, namely: Mysore, Goldfinger, and Imove. These species were strongly associated with B and AB genomes. Accessions/cultivars with unknown BSV sequences will be further characterized.

Keywords: banana streak virus, endogenous BSV, episomal BSV, multiplex PCR

BS - 37

INFLUENCE OF LP-3G3 PROBIOTIC FOODS ON THE DISTAL GUT BACTERIAL FLORA OF MICE (*Mus musculus* L.)

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Pediococcus acidilactici (LP-3G3), a lactic acid bacterium, can be formulated into functional foods for preventing and managing lifestyle-associated diseases such as obesity. To initially assess the efficacy and safety of LP-3G3 as a probiotic, the influence of LP-3G3 probiotic foods on the distal gut (colon) bacterial flora of mice was determined. Locally formulated chocolate bar and drink containing LP-3G3, Yakult probiotic drink containing *Lactobacillus casei* strain Shirota (LcS) and Orlistat[®] were orally administered to groups of standard diet (SD)-fed and high-fat diet (HFD)-fed BALB/C mice. The bacterial profile was obtained through PCR and denaturing gradient gel electrophoresis (DGGE) and analyzed using Dice's index of similarity. The bacterial community members were identified through rDNA sequencing. Baseline distal gut bacterial diversity in HFD-fed mice was greater than the SD-fed group. Upon feeding with LP-3G3 chocolate bar, the distal gut bacterial flora of HFD-fed mice became less diverse. LP-3G3 chocolate-fed mice had more diverse distal gut bacterial flora compared to the Orlistat-treated and the untreated mice. LP-3G3 probiotic drink had a greater effect than Yakult in shifting the distal gut bacterial flora of HFD-fed mice to the SD-type. However, LP-3G3 probiotic drink and Yakult had little effect on the distal gut bacterial flora of SD-fed mice. Orlistat had a similar effect as Yakult in all treatment groups. *Pediococcus acidilactici* had a greater influence than *Lactobacillus casei* strain Shirota on the distal gut bacterial flora, inhibiting the growth of Firmicutes bacteria which increased in number due to a HFD.

Keywords: *P. acidilactici*, probiotics, gut bacteria, rDNA, DGGE

BS - 38

**KINETICS STUDY AND SUBSTRATE OPTIMIZATION OF
Lactobacillus plantarum BSBY BATCH FERMENTATION**

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An optimization study of modified medium was done to predict the optimum substrate compositions necessary for the growth of *Lactobacillus plantarum* BS. Using Central Composite Design, the medium compositions were varied with values ranging from 1.3-5% w/v total sugars, 0.6-2% w/v nitrogen, and 2-5% w/v yeast extract, respectively. Cheese whey was used as the carbon-source, soybean meal extract as the nitrogen-source. The optimum viable cell count (7.56696×10^7 CFU/mL) was obtained in media consisting of 1.3% total sugars, 2.0% nitrogen, and 5.0 % yeast extract. The three medium components used all had significant effects on the growth of *L. plantarum* BS. The growth kinetics of *L. plantarum* BS in cheese whey was also studied. Total sugar concentrations of 2.3%, 4.15%, and 6.0% w/v were prepared with 2.0% nitrogen and 5.0% yeast extract. Total sugar consumption, pH, and viable cell count were monitored at regular interval for 10 hours. Substrate consumption was highest at 4.15% total sugars where the bacteria had the highest viable cell count equal to 7.75×10^{10} CFU/mL, and even higher than the growth in de Man-Rogosa-Sharpe (MRS) broth, a defined media for lactic acid bacteria. pH dropped as fermentation time progressed. The kinetic model that best described the growth of *L. plantarum* BS in cheese whey was the Moser Model. The calculated kinetic parameters were μ_{\max} equal to 0.365557 hr^{-1} and K_s equal to 1429.698 mg/mL having R^2 equal to 0.97913.

Keywords: *Lactobacillus plantarum*, central composite design, growth kinetics

BS - 39

**MICROBIAL DECOMPOSITION OF LEAF LITTER ON THE
MANGROVES OF PUNTA SULONG, BALIANGAO,
MISAMIS OCCIDENTAL, WESTERN MINDANAO**

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This study was conducted in the mangroves of Punta Sulong, Misamis Occidental to: 1) determine and describe the physical properties of the leaf litter; 2) determine the microbial decomposition rate of mangrove leaf litter; 3) compare the rate of microbial decomposition of the two study stations; and 4) describe the site in terms of its substrate, pH, peat depth, rainfall and temperature. Two study stations were established in the mangroves of Punta Sulong, wherein each station was subdivided into two zones, front and back. Yellowing leaves of dominant and co-dominant species of mangroves were collected. A 1mm mesh nylon window screening net was used for the three-litterbag harvest within a period of 42 days. Leaf biomass was estimated wherein $ODW = [0.508 + 0.211] \times [\text{fresh wt.}]$. Results showed that as the number of days of decomposition increased, a change in the color of the leaves occurred, which is evidence that leaching had occurred. After several weeks these were already fragmented to small pieces and others were highly disintegrated. Leaf thickness of *Rhizophora* leaves had a mean of 0.58mm and hardness with a mean of 17.88g. Leaf hardness showed significant correlation to microbial decay ($P=0.0241$). Leaf biomass loss was higher at the back zone which had 7.52g (20.73%) as compared to the front zone which had only 6.50g total loss (19.31%). Station 1 (along Kawayan River) showed a higher biomass loss with a mean of 8.00g (23.98%) as compared to Station 2 (along Dioyo River) which had a mean total loss of 6.12g (16.72). Rate of biomass loss in Station 1 was also higher (0.19g ODW/day) than Station 2 with only 0.14g ODW/day. The rate of biomass loss in both stations of the mangroves of Punta Sulong, Misamis Occidental was 0.17g ODW/day.

Keywords: microbial decomposition, leaf litter, mangroves, Punta Sulong, Baliangao, Misamis Occidental

BS - 40

**MICROBIAL EXTRACTION OF PECTIN FROM
CALAMANSI PEEL**

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Pectin is a widely used polysaccharide due to its gelling and emulsion stabilizing properties. Calamansi peel, a waste product was explored as source of pectin in the study. Current practice in the extraction of pectin involves the use of heat and acid which is not safe for the environment. Thus, extraction of pectin by microbial means was explored. *Saccharomyces cerevisiae* BIOTECH 2030 exhibited highest protopectin solubilising activity (1.2g crude dried pectin/50ml filtrate). Fermentation conditions for microbial extraction of pectin from calamansi peel using *S. cerevisiae* 2030 include substrate concentration of 7 g peel per 80 mL water, inoculum age and level of 24 hrs and 5%, respectively. The microbially extracted pectin from calamansi peel had galacturonic acid content comparable with commercial pectin.

Keywords: calamansi peel, pectin, yeast, protopectin , fermentation

BS - 41

PHILIPPINE *Bacillus* AS AGENT OF CONTROL AGAINST *Ralstonia solanacearum*

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Ralstonia solanacearum (Smith) (formerly called *Pseudomonas solanacearum*) is a devastating soil-borne pathogen that is widely distributed and considered a major limiting factor in the production of many crop plants around the world. *R. solanacearum* is the causal agent of bacterial wilt, a very destructive disease in tomato, banana, potato, eggplant, and some ornamentals. Although much progress has been made in the understanding of the biology of the pathogen, the disease continues to pose serious problems to farmers, particularly in the tropical and sub-tropical countries, like the Philippines. Application of chemical pesticide is still the method of control of *R. solanacearum*. But because of the health and environmental concerns, the use of microbes to control has been very significant in recent years. This research involved isolation and screening of *Bacillus* as control agent against bacterial wilt-causing pathogen, *Ralstonia solanacearum*. A total of 400 *Bacillus* isolates from soil were screened for their antibacterial activity against *R. solanacearum* (E. F. Smith) using agar plug method. Among the 135 *Bacillus* isolates that inhibited *R. solanacearum*, isolates BB142 and BC 152 showed the highest *in vitro* inhibition at 28.7mm and 32.8mm, respectively. Greenhouse experiment showed that the mixed antagonists, BB 142 and BC 152 proved to be the most effective against *R. solanacearum*. Infected tomato plants, root-dipped in mixture of both BB 142 and BC 152 isolates showed no incidence of wilting even after 16 days of inoculation. Biochemical and morphological characterization identified the *Bacillus* isolates BB142 and BC 152, as *Bacillus subtilis*.

Keywords: *Ralstonia solanacearum*, biocontrol, bacterial wilt, *Bacillus*, agent of control

BS - 42

**REPETITIVE SEQUENCE-BASED PCR AND
IDENTIFICATION OF DNA MARKERS IN SELECTED
LACTIC ACID BACTERIA STRAINS**

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Repetitive sequence-based PCR were done to determine the DNA profiles of bacterial isolates with beneficial properties and to identify species and strain-specific markers. A total of 22 bands were scored in nine *Lactobacillus plantarum* isolates and *Lb. plantarum* ATCC 8014. Repetitive Extragenic Palindromic (REP) primers generated nine types of profiles in ten *Lb. plantarum* strains demonstrating the usefulness of REP sequences in strain differentiation. Two bands, with sizes ranging from 0.38 to 0.55 kb, were present in all strains tested and thus could serve as markers for the species. Isolate F39 obtained from fermented guava leaves was most closely similar to two isolates from a fermented meat product. However, no strain specific marker for F39 was observed in its REP profile. Though highly similar profiles were observed between *Lb. fermentum* FM7 and *Lb. fermentum* F36, a 1.4 kb band differentiated F36 from FM7. Compared to REP sequences, the distribution of Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences in *Lb. plantarum* strains was conserved, with a total of six common bands in all *Lb. plantarum* strains tested. Among the three repetitive sequence-based PCR, BOX A1R demonstrated the most conserved DNA profile in *Lb. plantarum*. No unique nor differential DNA marker could be detected for F39 in its BOX profile. Its usefulness in *Lb. plantarum* species identification relies on the presence of five bands. On the other hand, for *Lb. fermentum* F36, a very intense 3.3 kb band, which was not present in FM7, is a potential DNA marker for the strain.

Keywords: rep-PCR, DNA marker, lactic acid bacteria, *Lactobacillus plantarum*, *Lactobacillus fermentum*

BS - 43

**TERATOGENIC POTENTIAL OF CYANOBACTERIUM
(*Nostoc commune*) ON WHITE MICE (*Mus musculus*)**

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This study focused on the teratogenic potential of cyanobacterium *Nostoc commune* on white mice (*Mus musculus*). The dominant lethal test was employed through feeding using 20%, 40%, and 80% of *N. commune*. Sixteen mature females and eight mature males were randomly assigned into three experimental groups and a control. The female mice were sacrificed on the 19th day of pregnancy. Dunnett's Test and one-way ANOVA were used for the statistical analysis. It was found out that *Nostoc commune* has teratogenic potential. Eighty percent yielded the highest %dead implants, %females with resorption, and lowest gestation index, fertility index, and implantation index. There was a decrease in gestation index, implantation index, fertility index, and an increase in %dead implants and %females with resorption. Statistically, there was no significant difference on gestation index and implantation index between the experimental and control groups, but there was a significant difference in the fertility index, %dead implants, and %females with resorption.

Keywords: teratogen, teratogenicity, *Nostoc*, cyanobacterium, white mouse

BS - 44

**THE EFFECT OF STORAGE TEMPERATURE (4 °C) ON THE
ACTIVITY OF ISOLATED BACTERIOPHAGE AGAINST
Salmonella spp. ISOLATED FROM RAW CHICKEN**

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Salmonella is a gram-negative, rod-shaped, motile, non-spore forming bacterium which causes diarrhea to septicemia that is usually contracted through consumption of contaminated foods. Outbreaks of salmonellosis are a perennial problem especially in the poultry industry where alternatives to chemicals to battle this bacteria have been investigated. Bacteriophages, which are capable of lysing *Salmonella* by penetrating through their cell membrane and disrupting their metabolic processes have been explored as an alternative, considering they are safer to use. *Salmonella* Havana was isolated from raw chickens, following the ISO method 6579:2002. A bacteriophage, designated as Bacteriophage A3CE, capable of lysing *Salmonella* Havana was isolated from soil. When the ratio of bacteriophage to *Salmonella* Havana was 10 or more (MOI >10), the number of *Salmonella* Havana was reduced by > 90%, 6 hours after the bacteriophage reached its maximum burst size at room temperature. The same experiment was conducted at 4°C. Results showed that there is no significant difference between the activity of the isolated bacteriophage at room temperature and at 4 °C (P > 0.05). Thus, Phage A3CE was effective in reducing the amount of *Salmonella* Havana at both temperature conditions which makes it a promising alternative agent against the said bacterium.

Keywords: salmonellosis, *Salmonella*, raw chicken, bacteriophage, storage temperature

BS - 45

THE IMMUNOMODULATORY EFFECT OF *Exiguobacteria* sp. ISOLATED FROM SHRIMP GUT ON GROWTH AND SURVIVAL OF *Macrobrachium rosenbergii* CHALLENGED WITH SHRIMP PATHOGENS

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The use of alternative disease control measures such as the utilization of probiotics is widely studied nowadays for efficient management of shrimp aquaculture. This study examined the effect of the pre-isolated bacteria on the growth and immune responses of *Macrobrachium rosenbergii* against viral (White Spot Syndrome Virus) and bacterial (*Vibrio* spp.) pathogens. In addition, the median lethal dosage (LD₅₀) of WSSV and *Vibrio* spp. was determined. Furthermore, the analysis on *Exiguobacteria* sp. as potential probiotic was done through the determination and comparison the immune parameters such as the weight, survival, Total Hemocyte Count, and Phenoloxidase Activity of the experimental and controlled samples. The treatments included the following: *Exiguobacteria* sp. and *Bacillus* sp. separately incorporated to commercial shrimp feed, both of which were fed for 14 days and 21 days, and commercial feed with no addition of probiotics (control). Results showed that both BS (*Bacillus* sp.) and Exi (*Exiguobacteria* sp.) treatments affected ($P < 0.05$) the growth and exhibited increase in survival of *M. rosenbergii* as compared to that of the control group. This investigation suggested that *Bacillus* sp. and *Exiguobacteria* sp. are affective probiotics in rearing *M. rosenbergii* based on enhancement of growth and survival rate against *Vibrio* spp.

Keywords: probiotics, *Macrobrachium rosenbergii*, *Exiguobacteria* sp., *Bacillus* sp., white spot syndrome virus

BS - 46

JACKFRUIT BRONZING DISEASE IN THE PHILIPPINES IS CAUSED BY *Pantoeastewartii* (Smith) Mergaert *et al.*

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Jackfruit bronzing, an unreported disease affecting jackfruit, is characterized by yellowish-orange to reddish discoloration of the affected pulps and rags of the fruit. The disease etiology is the scope of this study. The pathogen was isolated from infected jackfruit, and pathogenicity was conducted. The pathogen was characterized and identified based on its cultural and morphological characteristics, staining reactions, physiological, biochemical characteristics, and other plant inoculations. Initial identification was confirmed through DNA analysis through polymerase chain reaction using *Pantoeastewartii*-specific primers. The bacterium produced a yellow pigment in culture; Gram negative; non-motile; slightly pleomorphic; facultatively anaerobic short-rods; measuring 1-2 um in length; catalase positive; able to hydrolyze gelatin and starch but not tween80; produced acid from glucose, galactose, fructose, sucrose and maltose but not from lactose; did not produce hypersensitivity to tobacco; caused pits but not soft rot on potato discs; and infected corn producing the same symptom as bacterial wilt or Stewart's disease. PCR analysis confirmed the cause as *Pantoeastewartii* or *Pantoeastewartii* subsp. *stewartii* (Smith) Mergaert *et al.*, (formerly *Erwiniastewartii*) (Smith) Dye, the same bacterium that causes bacterial wilt or Stewart's disease of corn.

Keywords: bronzing, disease, etiology, Jackfruit, *Pantoeastewartii*

BS - 47

ACTINOMYCETES AS BIOCONTROL AGENT AGAINST PANAMA DISEASE CAUSING *Fusarium oxysporum*

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Fusarium oxysporum sp *cubense* tropical race 4 (Foc TR4) is a highly virulent form of Foc that attacks primarily the Cavendish variety of banana, a major export product of the Philippines. The study sought to evaluate the potential of actinomycetes to control Foc. Actinomycetes for biocontrol were isolated from soil samples in mangrove areas in Quezon and Bataan. Screening for biocontrol activity was initially done by agar plug and cup cylinder bioassays against Luzon isolates of Foc. Selected best isolates were then assayed against pure isolate of Foc TR4 that was obtained from Lapanday Foods Corporation through the University of Southeastern Philippines, Tagum City, Davao Oriental. Eighty-two out of a total of 199 actinomycetes isolated showed activity against the Luzon isolate of *F. oxysporum*. Six of these had e"18.0 mm zone of inhibition by agar plug assay. Five of these isolates gave high activity by cup cylinder assay with isolates AQ6, AQ30 and AQ121 as the best three isolates inhibiting *F. oxysporum* by 21.0 mm, 22.0 mm and 20.5 mm, respectively. The three best isolates selected also showed good biocontrol activity against Foc TR4. Bioassay of AQ6, AQ30 and AQ121 gave 24.6 mm, 20.2 mm and 19.0 mm zones of inhibition, respectively in the agar plug assay and 8.3 mm, 12.0 mm and 13.7 mm, respectively for the cup cylinder assay. Combinations of the three isolates yielded an inhibition of 13.5mm by cylinder cup assay. The present study showed the effectivity of actinomycetes in controlling Foc TR4 *in vitro*. These findings led to the formulation of biocontrol using actinomycetes for greenhouse and field tests to manage the disease and prevent further spread of Foc TR4.

Keywords: actinomycetes, biocontrol agents, *Fusarium oxysporum*, Panama disease, agar plug assay

BS - 48

BIOMASS DEGRADATION ACTIVITY OF FUNGI ISOLATED FROM SUGARCANE BAGASSE UNDER SOLID STATE FERMENTATION

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Conversion of lignocellulosic biomass to ethanol as biofuel is a viable option to address problems of energy economics and environmental concerns. Residual non-food biomass from the agricultural sector like sweet sorghum serves as a promising alternative feedstock for ethanol production. In nature, fungi contribute significantly to the decay of biomass by producing lignocellulolytic enzymes. In this study, two strains of fungi isolated from sugarcane bagasse were evaluated for their biodegradation activity on sweet sorghum bagasse under solid state fermentation. Two fungal strains were isolated from decomposing sugarcane bagasse. One fungal strain (F1) exhibited the characteristics of an ascomycete, having a light green color with loose septate conidiophores. The other strain (F2) had spores distinct from basidiomycetes. Stalks of sweet sorghum were collected from the MMSU Sweet Sorghum project. Samples were dried, cut, and ground to 40 mesh. Samples were inoculated with individual spores of the two fungi. Incubation was carried out at 27°C on a rotary shaker (160 rev/min) for 30 days. Analyses of the biomass constituents of the extractive-free stalks - cellulose, hemicellulose, and lignin - was carried out before and after, following the TAPPI standard procedures. Treatment inoculated with F1 showed a decrease of cellulose, hemicellulose, and lignin at 19.30%, 3.67%, 1.03%, respectively, while F2 treated samples showed a decrease in cellulose (12.46%), hemicellulose (4.55%), and lignin (3.26%). Both fungi showed potential for biodegradation of sweet sorghum bagasse.

Keywords: lignocellulosic biomass, ethanol, sweet sorghum, fungi, biodegradation

BS - 49

BIOMASS DEGRADATION ACTIVITY OF INDIGENOUS FUNGI ISOLATED FROM BANANA STALK UNDER SOLID STATE FERMENTATION

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In solid state fermentation, lignocellulosic biomass is converted to carbohydrates which can be further fermented for the production of ethanol. The main purpose of this study was to determine the biodegradation activity of two indigenous fungi to banana using solid state fermentation. The fungi used in this study were isolated from decomposing sugarcane bagasse. One of the fungi (F1) used in this study has the characteristics of an Ascomycete. It is light green and its conidiophores are septate and are loosely branched. Its conidia are smooth, pale green and spherical in shape. The other fungus (F2) used in this study showed the characteristics of Basidiomycota spores. Banana pseudostem was ground to 40 mesh size. The samples were then inoculated with the spores of the two fungi using standard protocols in inoculating fungi for Solid State Fermentation. After 30 days of incubation, the lignocellulosic biomass of the samples were analyzed using TAPPI standard procedures. The samples treated with F1 showed a decrease in lignin, cellulose and hemicellulose at 1.05%, 18.70%, and 4.2%, respectively. The samples treated with F2 showed a decrease in lignin, cellulose and hemicellulose at 2.09%, 16.34%, and 2.2%, respectively. Consequently, both the fungi showed biodegradation activity on the banana pseudostem.

Keywords: solid state fermentation, banana, fungi, biodegradation, basidiomycota, ascomycota, ethanol

BS - 50

KINETICS STUDY AND SUBSTRATE OPTIMIZATION FOR THE GROWTH OF *Pediococcus acidilactici* 4E5 BY BATCH FERMENTATION

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Pediococcus acidilactici 4E5 isolated from “burong tilapia” is a bacteriocin-producing microorganism which inhibits growth of pathogenic microorganism which can be used in biopreservation processes. For large scale production, alternative low-cost nutrients as media components were investigated. Cheese whey, soybean meal extract and baker’s yeasts were used as carbon-source, nitrogen-source and yeast extract-substitute, respectively. The Central Composite Design was used to predict the optimum growth of *P. acidilactici* 4E5 in the modified low-cost media. The media formulation consisting of 1.3% carbon, 2% nitrogen and 5% yeast extract gave the highest average viable cell count of 8.875×10^7 CFU/mL. Having obtained the best media formulation for the growth of 4E5, kinetics study of 4E5 in low-cost growth medium was done. Media for kinetic study consisted of sugarcane molasses as carbon source at concentrations (1.3%, 3.15%, and 5%) with nitrogen (2%) and yeast extract (5%). Parameters such as pH, viable cell count, % titratable acidity, and total sugar were determined during the 12-hour fermentation. Results showed pH and total sugar concentrations decreased with fermentation time. An indication of the growth of 4E5 and subsequently, lowering of pH due to presence of lactic acid in the medium. The specific growth rate was determined. The growth of *P. acidilactici* 4E5 was best described by the Moser Model yielding a μ_{max} value of 0.173319 hr^{-1} and K_s value equal to 4744.6141 g/mL .

Keywords: *Pediococcus acidilactici*, central composite design, fermentation, kinetic study

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**FUNCTIONALITY OF *Lentinus tigrinus* (Bull.) Fr.,
AN EDIBLE BASIDIOMYCETE FROM THE PHILIPPINES**

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Filipinos are now searching for organic foods with important nutrients and multifunctional activities, which are termed as nutraceuticals. The bioactive components of edible mushrooms have emerged as natural sources of compounds that are antioxidant, antibacterial, anti-hypercholesterolemic, anti-diabetic, antiviral, and antifungal. Our team has been continually searching for Philippine wild edible mushrooms with nutraceutical potential. One of the candidates is a white wood-rotting basidiomycete, the *Lentinus tigrinus* (Bull.) Fr. Recently, the optimum cultural conditions and production technology for this mushroom were established. In this study, we determined the antibacterial and hypoglycemic activities of *L. tigrinus*. *In vitro* antibacterial assay showed that the ethanolic extract of fruiting body and the immobilized secondary mycelia had high antibacterial activity against *Staphylococcus aureus*. The administration of lyophilized hot water extract of the fruiting body (both 100 mg/kg and 250 mg/kg dosages) to diabetic-induced mice significantly lowered the glucose level by 26.9% on the third week, which was comparable to the anti-diabetic agent glibenclamide. With these significant biological properties, *L. tigrinus* can be considered as natural source of safe nutraceutical.

Keywords: *Lentinus*, nutraceutical, functionality, mushroom, diabetes

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**NUTRITIONAL AND PHYSICAL CULTURE CONDITIONS
FOR MYCELIAL GROWTH AND FRUITING BODY
PRODUCTION OF TWO STRAINS OF
Ganoderma lucidum (Leys.) Karst**

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Ganoderma lucidum (Leys.) Karst belongs to the group of Basidiomycetes. The fruiting body is glossy reddish- orange to brownish black color with definite stalk attach to the cap. It is usually found growing on dead trunks of trees in the forest. This study evaluated the optimum culture conditions for secondary mycelial growth and fruiting body performance of the two strains (*Munoz* and *Cuyapo*) of *G. lucidum*, with special reference to the influence of nutritional factors (different indigenous culture media), physical factors (pH, aeration, illumination and temperature), locally available granulated spawning materials (sorghum seeds, palay grains and feed conditioner), and rice straw based substrate formulation. Results revealed that regardless of the strain type, the most suitable culture conditions for mycelial growth of *G. lucidum* was coconut water gulaman at pH 7, incubated in unsealed plates, either dark or lighted at room temperature condition. Corn grit produced the very thick mycelia growth and shortest incubation period with a mean of 6.0 days. Furthermore, 7 parts rice straw + 3 parts saw dust combination produced the heaviest fruiting bodies (15.85 g) and highest biological efficiency (4.70%). The *Munoz* strain produced heavier fruiting bodies and higher biological efficiency than *Cuyapo* strain.

Keywords: *Ganoderma lucidum*, *Munoz* and *Cuyapo* strains, indigenous culture media, physical factors, secondary mycelia

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**RADICAL SCAVENGING ACTIVITIES AND TLC-GUIDED
BIOAUTOGRAPHY OF THE SC-CO₂ EXTRACT OF
*Ganoderma applanatum***

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Supercritical carbon dioxide (SC-CO₂) extracts from the fruiting bodies of *Ganoderma applanatum* were characterized by reverse phase high performance liquid chromatography (HPLC) and fourier transform infrared spectroscopy (FTIR), and its antioxidant compounds were determined through DPPH Radical Scavenging Assay and TLC-guided bioautography. The extracts were found to be essential oils in 10 mPa and secondary metabolites in 20 & 30 mPa. The DPPH assay, which determines radical scavenging activity, showed that the SC-CO₂ extracts have antioxidant activity in the following order: 10 mPa < 30 mPa < 20 mPa < ascorbic acid. Results of TLC-bioautography using 2.54 mM DPPH showed that the compounds have antioxidant property through the changed in color of the extracts in the TLC plate. These results suggest that the extracts serve as a source of compounds with radical scavenging activity that can be used as antioxidants aside from its traditional uses.

Keywords: *Ganoderma applanatum*, SC-CO₂ extracts, Reverse-phase HPLC, FTIR, DPPH radical scavenging assay, TLC-guided bioautography

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**SYNERGISTIC EFFECT OF CHEMICAL AND
ULTRAVIOLET IRRADIATION ON THE
BIODEGRADATION OF LOW DENSITY POLYETHYLENE
(LDPE) BY *Aspergillus oryzae***

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The ability of *Aspergillus oryzae* to degrade low-density polyethylene (LDPE) was tested with chemical and ultraviolet (UV) radiation pre-treatments. The degree of degradation was measured after 45 days incubation in Zchapek's broth medium with *Aspergillus oryzae*. LDPE plastic strips (1 in x 4 in) were soaked in 0.01% potassium permanganate solution for 48 hours and exposed to UV-A radiation for 120 hours. Elongation length, maximum force required to tear plastic and physical changes of the plastic material were noted. Results show a synergistic effect of chemical and UV pre-treatment, implicating the highest degradation result with 43.56% reduction of the maximum force needed to break the material and 84.26% reduction in the elongation length at break. With UV pre-treatment alone, there was only 35.81% reduction in maximum force and 70.02% reduction in elongation length. With chemical pre-treatment alone, a reduction of 31.88% in maximum force and 76.96% in elongation length were observed. Elongation length at break had been statistically proven significant at 5% level. Based on the analysis, both the chemical treatment ($p = 0.011$) and UV irradiation ($p = 0.019$) have a direct effect on the elongation length of the samples. It is therefore apparent that LDPE can be degraded through fungal exposure, provided that sufficient chemical and mechanical pre-treatments are administered.

Keywords: biodegradation, *Aspergillus oryzae*, low-density polyethylene (LDPE), UV irradiation, potassium permanganate

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**FUNGAL ENDOPHYTES FROM MANGROVE
LEAVES AND STEMS: SOURCES OF METABOLITES
WITH ANTIMICROBIAL, ANTIOXIDANT,
AND CYTOTOXIC ACTIVITIES**

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The search for new drugs recently taps fungal endophytes associated with tropical plants. In this study, fungal endophytes from stems and leaves of Philippine mangroves (MFE) were grown *in vitro* for the mass production of bioactive secondary metabolites. Eighteen MFE crude culture extracts were then tested for their antimicrobial, antioxidant, and cytotoxic activities. Results showed that six MFE exhibited inhibitory activities (11-16 mm ZOI) against at least one of the nine test microorganisms. The crude culture extracts were more active against gram-positive than gram-negative bacteria as shown by the paper disk diffusion assay. Cytotoxic activity using the brine shrimp (*Artemmia salina*) lethality assay showed that six of the MFE killed at least 50% of the test organism at a concentration of 100 µg. One MFE exhibited 100% brine shrimp lethality. Of the seven MFE extracts tested for their antioxidant activity using DPPH assay, only four showed 40-52% radical scavenging activity. Our research study highlighted the potential of fungal endophytes from Philippine mangroves as sources of metabolites with pharmaceutical importance and for novel drug discovery.

Keywords: bioassay, biological activities, *in vitro* culture, mangrove fungi, secondary metabolites

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***Lentinus squarrosulus* (MONT.) SINGER AND *Polyporus grammacephalus* BERK.: NEWLY DOMESTICATED, WILD EDIBLE MACROFUNGI FROM THE PHILIPPINES**

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Lentinus squarrosulus (Mont.) Singer and *Polyporus grammacephalus* Berk. are wild, edible macrofungi utilized as food by the indigenous Aeta tribes in Botolan, Zambales. Domestication of these wild edible mushrooms is thus necessary to render these fungi available all year round for food and other purposes. In this study, the two edible macrofungi were grown *in vitro* using different indigenous culture media, grain spawning materials, and combinations of rice straw and sawdust formulations for its secondary mycelial growth and mass production of fruiting bodies. Results showed that secondary mycelial growth was observed best on coconut water-*gulaman* medium. Sorghum seeds and/or corn grits also yielded very luxuriant mycelial growth at shortest incubation period of 6 days for *L. squarrosulus* and 7 days for *P. grammacephalus*. Highest biological efficiency (7.83%) was noted in 100:400 rice straw:sawdust formulation for *L. squarrosulus*. In contrast, highest biological efficiency (2.91%) was recorded for *P. grammacephalus* at 400:100 rice straw:sawdust formulation. The macrofungi reported in the study are new additions to the record of successfully domesticated wild, edible macrofungi in the Philippines.

Keywords: *in vitro* cultivation, mycelial growth, indigenous media, grain spawns, fruiting body production