BIOLOGICAL SCIENCES

BSD No. 1

ESTABLISHING A NEW CODON PREFERENCE TABLE FOR THE COCONUT (*Cocos Nucifera* L.)

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The triplet codes of 18 sequences derived from ten genes isolated from coconut (*Cocos nucifera* L.) were tallied. Relative frequency percentages of the individual codons encoding each amino acid were calculated. The highest percentage of the codon for each amino acid was chosen as that amino acid's most preferred codon. Comparative analysis to determine whether a change of codon preference and degeneracy exists was done between the published codon preference table (Nakamura et al, 2000) and from a new one generated from the sequence data of the coconut genes. A similar analysis was done for the sequence data of the genes derived from the normal and the makapuno phenotypes.

Analysis between the published codon preference table and the table derived from this study showed that 30% of the amino acids retained the existing published preference codon, 35% of the amino acids prefer a non-degenerate codon while 35% had a change of codon preference especially at the third nucleotide position of the codon. Moreover, arginine prefers a degenerate codon with an additional change at the first nucleotide position over the reported codon preference.

Analysis between the genes derived from the normal and makapuno phenotypes shows that 70% of the amino acids utilize the same codon across the two phenotypes; 5% has a distinction with the normal coconut phenotype preferring a degenerate codon while its makapuno counterpart utilizes a non-degenerate codon; and, 25% of the amino acids had a change in codon preference

between the two phenotypes.

This is a pioneering study in establishing the coconut codon preference table, a molecular biology tool much-needed by researchers in gene discovery and other applications in instances where molecular biology data is limiting.

Keywords: codon preference, coconut, *Cocos nucifera* L.

BSD No. 2

DETECTING ISOFORMS OF GENES INVOLVED IN FATTY ACID SYNTHESIS IN COCONUT (*Cocos nucifera* L.)

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The RACE (Randomly Amplified cDNA Ends) method is a very useful and accurate technique in detecting isoforms of various genes. In coconut, several genes are involved in fatty acid synthesis and among them are: phosphatidic acid phosphatase (PAP), acyl carrier protein (ACP) and beta-keto acyl (ACP) synthase 3 (KAS 3). To check for the presence of isoforms of each gene at the 4, 5 and 6 month old coconut endosperms, the RACE method was used.

Forward primers of both ACP and KAS 3 were designed from the highly conserved amino acid region based on previous publications. The forward primer of PAP was designed based on multiple sequence alignment of known PAP sequences. Reverse primer used for all three genes was provided by the RACE kit.

Initially, cold-start PCR was used and a 300 bp band was obtained for ACP in the 4 mo old coconut endosperm. No distinguishable band was obtained for PAP and KAS 3. Both Pap and KAS 3 were further subjected to an improvised hot-start and touchdown PCR, which yielded three bands with sizes 700 bp, 500 bp and 400 bp, respectively for PAP at the 6 mo. old coconut endosperm while two bands with sizes 725 bp and 425 bp, respectively were obtained for KAS 3 in both the 5 and 6 month old coconut endosperm.

The results obtained indicate the presence of each of the gene and their isoforms at varying ages of the coconut endosperm. Furthermore, the present results are consistent with the results obtained using the coconut cDNA library.

Keywords: Randomly Amplified cDNA Ends (RACE), phosphatidic acid phosphatase (PAP), acyl carrier protein (ACP), â-ketoacyl (ACP) synthase 3 (KAS 3), isoforms, PCR

BSD No. 3

COCONUT (*Cocos Nucifera* L.) EXPRESSES *GUS* IN SELECTED PLANT PARTS

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This study stems from our research to develop a transient expression analysis system using embryogenic callus derived from zygotic embryos of coconut. The transient expression analysis is necessary to establish some physical parameters such as distance (between the gun and the target tissue) and pressure of the helium gas in the optimization of a particle gun bombardment system for transformation in coconut. pBI121 is a binary vector which contains the *GUS* gene and is driven by a 35S CaMV promoter. This 13 kb plasmid was propagated in *E. coli*, and the isolated plasmids were purified using a commercial purification kit. The purified plasmids were coated unto tungsten and bombarded directly to embryogenic callus derived from zygotic embryos of coconut using a particle inflow device. After particle bombardment, expression of the *GUS* gene was assayed using standard histochemical staining protocols. *GUS* expression through the formation of blue spots scattered randomly was observed in embryogenic calli bombarded with pBI121-coated tungsten and with tungsten only. Contamination was ruled out by including some controls in the experiments. Different portions of the coconut (endosperm, embryo, young leaves, young stem, haustorium) were histochemically assayed for endogenous *GUS* or GUS-like activities and the immature endosperm, young stem and haustorium showed positive reaction for GUS activity. These results show that coconut contain endogenous GUS activity and therefore *GUS* may not a suitable reporter gene assay for transient expression analysis in coconut.

Keywords: coconut, *Cocos nucifera* L., *GUS*, transformation

BSD No. 4

BULKED SEGREGANT ANALYSIS (BSA): A RAPID PROCEDURE FOR IDENTIFYING AFLP MARKERS IN THE SPECIFIC REGIONS OF THE RICE TUNGRO SPHERICAL-VIRUS DISEASE RESISTANCE GENE LOCUS IN RICE (*Oryza sativa* L.)

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The most important viral disease that causes yellowing and stunting in rice plants, inflicting heavy loses on rice is the Tungro virus disease. The disease is caused by a composite of two viruses, the rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). The green leafhopper (*Nephotettix virescens* Distans) is the major transmitter of the rice tungro virus disease. In this study, genetic mapping of the tungro spherical virus (RTSV) resistance gene was undertaken using the bulked segregant analysis (BSA). The BSA involves comparing two pooled DNA samples from a segregating population derived from a single cross, delineating the R gene to a narrow region through the identification of candidate Amplified Fragment Length Polymorphism (AFLP) markers.

In the genetic analysis, the F_2 and F_3 populations from the cross between TI-11-8, a TN1 line with introgressed R gene from ARC11554, and a susceptible line R4-40/RCN13-19-94 were grown for DNA isolation and tungro phenotypic screening. Two hundred eighty-six F_3 families were evaluated for tungro reaction through ELISA after RTSV inoculation. A bulked segregant analysis for amplified fragment length polymorphism (AFLP) was performed on the 16 highly resistant and 16 highly susceptible F_2 plants that were identified based on the ELISA scores of their corresponding F_3 families. The AFLP profile of the R and S pools and the four parentals were compared in each of the forty pairs of Pst1 and Mse1 primers as well as forty pairs of Eco R1 and Mse1 primers used. Seven polymorphic bands were identified for Pst1/Mse1 primer combinations while five were identified for the EcoR1 and Mse1 primer pairs. Hence, based on the mapping results these AFLP markers may lie within 0.3 cM from the R gene.

Keywords: Tungro, AFLP, bulked segregant analysis, green leafhoppers, markers

BSD No. 5

MOLECULAR ANALYSIS OF MITOCHONDRIAL GENOMIC VARIATION BETWEEN CYTOPLASMIC MALE STERILE (CMS) AND FERTILE LINES OF MESTIZO HYBRID RICE

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This study analyzed the genetic polymorphism between the cytoplasmic male sterile (CMS) and maintainer lines of Mestizo hybrid rice. Total genomic DNA was isolated from IR58025A and IR58025B, the CMS and maintainer lines, respectively. The mini-prep CTAB DNA isolation method was employed to extract and purify high quality DNA from the two rice varieties. Five PCR primers based on the atp6- ORF region of Bo- type mitochondrial genome was used to amplify mitochondrial DNA using total genomic DNA as template. PCR products were size- separated in 1% agarose gel electrophoresis. Variation in the PCR profiles (DNA fingerprints) between the A and B line was visualized and documented after ethidium bromide staining. Polymorphism was only detected in the PCR products with mit1 as primer. No polymorphism was detected in PCR products with mit2- mit5 as primers. It can be said that mitochondrial DNA can be used to detect variation in the A and B lines of Mestizo hybrid rice. It is suggested that more primers must be used to detect more polymorphism.

Keywords: genetic polymorphism, CMS, maintainer line, PCR, primers, mitochondrial genome, electrophoresis

BSD No. 6

HIGH THROUGHPUT SCREENING OF THE BACTERIAL ARTIFICIAL CHROMOSOME (BAC) LIBRARY USING THE THERMAL ASSYMETRIC INTERLACED PCR (TAIL-PCR) FOR THE PHYSICAL MAPPING OF TUNGRO SPHERICAL VIRUS RESISTANCE GENE IN RICE (*Oryza sativa* L.)

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Tungro is a viral disease that inflicts heavy loses on rice and is caused by rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). It is transmitted by the green leafhopper (*Nephotettix virescens* Distans). In this study, physical mapping was conducted to identify clones that will guide the map-based cloning of the resistance (R) gene identified in ARC 11554 to RTSV, the primary causal organism.

Two rice BAC libraries were screened using the thermal asymmetric interlaced PCR (TAIL-PCR). The TAIL-PCR has been developed for the isolation and amplification of insert end sequences of BAC clones. The TAIL-PCR strategy used three nested specific primers in successive reactions together with a shorter arbitrary degenerate (AD) primers so that the relative amplification efficiencies of specific and non-specific products can be thermally controlled. Probes used for library screening were derived from the previously mapped RFLP markers around the tungro R gene. Marker CDO456 bound to BAC4705, marker C708 hybridized to BAC 17N19, and marker CDO783 cleaved to BAC 19P8. Recurrent BAC end isolation by TAIL-PCR and library screening identified 11 more clones at the CDO456 locus, 13 more BAC clones at the C708 locus, and 4 more clones at the CDO783 locus. The selected BACs at C708 have an average insert size of 75 kb ranging from 35 to 105 kb as determined by pulse-field gel electrophoresis of the *Not* 1 digest. Preliminary assembly of the clones suggests a contig size of 190 to 335 kb. The advance toward the *R* gene from the C708 locus will be assessed based on the genetic mapping of the contig ends. Once cloned, the gene is envisioned to be utilized in the rapid development of tungro resistant varieties through genetic engineering.

Keywords: BAC, cloning, contigs, library, physical mapping, resistance, tungro

BSD No. 7

GENE DISCOVERY FOR PEST RESISTANCE IN CORN: BACTERIAL AND INSECT-GUT SPECIFIC CHITINASES

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Transgenic plant technology can be a useful tool in the development of resistant crops by introducing novel resistance genes into plant species. To date, two main strategies for the generation of insect-resistant plants have been employed. One approach is to use the entomicidal bacterium *Bacillus thuringiensis* (Bt) as a source of resistance genes and the other is to deploy other insect-resistance genes present in other organisms.

Chitinases are hydrolytic enzymes that can degrade the peritrophic membranes of larval midguts. Genes encoding chitinases could be used for stacking with the Bt gene. At least seven isoforms of the chitinase gene were isolated and cloned from a local strain of *Serratia marcescens*, an enteric insect pathogen. Partial cDNAs corresponding to midgut-specific chitinase were also isolated from larval tissues of corn borer isolated in Los Banos by RT-PCR. One of the seven isoforms of the bacterial chitinase gene is already a full length gene and being characterized prior to stable transformation in corn. Based on sequence analysis, it is homologous to ChiA (a secreted extracellular chitinase).

Keywords: chitinase, corn, *Serratia marcescens*

BSD No. 8

CHITINASE PRODUCTION BY *Serratia marcescens* AND ITS POTENTIAL CONTROL OF THE SHEATH BLIGHT PATHOGEN OF CORN

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Plant pathogenic fungi like *Rhizoctonia solani*, a causal organism of sheath blight of corn creates a major problem in boosting agricultural production. The use of microbial products like the enzyme chitinase can be a promising alternative to lessen the incidence of fungal infestation and at the same time lessen environmental hazards brought about by chemical use. This study aimed to optimize the cultural conditions of the wild type strain of *Serratia marcescens* for increased chitinase production and to test its effectively against *R. solani*.

A chitinolytic wild type *S. marcescens* LPM42 BIOTECH 1749 was grown by batch fermentation in varied cultural conditions to produce chitinase. The amount of crude chitinase excreted in the culture medium was assessed turbidimetrically through its ability to release N-acetylglucosamine (GlcNAc) after reaction with colloidal chitin obtained from crab shells. Incubating the culture broth containing 5% (v/v) inoculum, 0.6% (v/v) colloidal chitin without Tween 80 at an initial pH of 7.0, for 48 hr at room temperature with continuous shaking (158 rpm) were the optimum conditions for chitinase production of S. marcescens (0.004 units/ml).

Laboratory experiments to control the growth of *R. solani* Kuhn showed that soaking the sorghum seeds coated with fungal mycelia for 3 to 5 hours in undiluted crude chitinase extract showed significant reduction in mycelial proliferation after 12 hr of incubation. Complete inhibition of mycelial development was observed after 12 hr soaking in undiluted crude chitinase extract. No growth was observed even when the incubation was extended for 48 hr. These results demonstrated the potential of using even the crude form of the chitinase enzyme for the biocontrol of *R. solani*.

Keywords: *Serratia marcescens*, *Rhizoctonia solani*, chitinase, chitin

BSD No. 9

MOLECULAR SYSTEMATIC STUDIES OF THE PHOTOSYNTHETIC RHIZOBIA ISOLATED FROM AESCHYNOMENE SPP. USING *nifH* AND *nodA* GENE SEQUENCES

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The rhizobia isolated from *Aeschynomene* species have the ability to produce photosynthetic pigment, aptly termed as the photosynthetic rhizobia. These photosynthetic species belong to the a-2 Proteobacteria and are phylogenetically related to the non-phototrophic *Bradyrhizobium* species and the phototrophic *Rhodopseudomonas palustris* based on 16S rRNA sequence analysis. Their very unique biological characters, possessing the nodule forming and the photosynthetic abilities of *B. japonicum* and *Rps. palustris*, respectively, have led us to think that the photosynthetic rhizobia are the "missing link" between the two species. As such, these bacteria are good models for studying the evolution of nodulation, nitrogen fixation and photosynthesis in the α-*Proteobacteria*.

In this study, we performed a phylogenetic analysis of the genes involved in nitrogen fixation (*nifH)* and nodulation (*nodA*) in comparison with that of the 16S rRNA and the intergenic spacer region (ITS) to investigate the possible origin and evolution of their unusual characteristics. The 16S rRNA and ITS phylogenies showed that the photosynthetic rhizobia are mainly monophyletic and closely related to *B. japonicum* and *Rps. palustris*. The *nifH* phylogeny placed the photosynthetic rhizobia in a monophyletic group with the strains of *B. japonicum*, but far from the *Rps. palustris* strains. The *nodA* from the photosynthetic rhizobia, on the other hand, were highly conserved and phylogenetically distant from those of other rhizobial species. These results suggest that the nitrogen fixing photosynthetic rhizobia may have evolved with the nitrogen fixing *B. japonicum* from a common ancestor, while their *nodA* gene might have been acquired from a different species in the latter part of their evolution. The ultimate conservation of their *nodA* gene from species grown in separate geographical regions also suggests that this gene might have co-evolved with its host plant.

Keywords: photosynthetic rhizobia, *Aeschynomene*, *nifH*, *nodA*, 16S rRNA, phylogeny

BSD No. 10

MONOMORPHICITY OF *RALSTONIA SOLANACEARUM* BANANA STRAINS IN THE PHILIPPINES

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Ralstonia solanacearum causes two distinct diseases in bananas, the moko and bugtok. There are considerable differences in the symptomatology, distribution and epidemiology of bugtok and moko diseases in the Philippines but this has been established to be due to difference in the varieties infected. Analysis of genetic variation using molecular biology methods have shown that banana strains in the Philippines are homogenous except for some strains wherein the difference is very minimal (Ilagan, 1996). However, isolates used were limited only to Mindanao, majority of which came from Davao and Bukidnon. There is, therefore, a need to verify whether new banana isolates from the Philippines will give the same DNA types.

Seventy-seven new banana isolates of *R. solanacearum* obtained from Davao, Sultan Kudarat and Iligan City were all pathogenic on tomato (Yellow Plum). Using primers 759/760 and M114 in polymerase chain reaction (PCR), the expected 281 bp and 2.28 Kb products of 759/760 and M114, respectively, were obtained confirming their being *R. solanacearum* and banana strains. PCR analysis using REP primers produced 10 to 15 bands ranging from 298 to 5090 bp. Of the 81 isolates, 73 were found to have genetic profiles similar to that of the reference strains Bu24W and MoD6. Seven new strains possessed an extra band at about 3563 bp. This band is completely distinct from the extra bands present in the RA-02 haplotype thus creating a new haplotype. Therefore, there are now three haplotypes in the population of banana strains. This difference, however, is still minimal confirming the monomorphicity of the banana strains. An abaca isolate tested produced a genetic profile that was completely distinct from the banana isolates of *Ralstonia solanacearum* using REP primers.

Keywords: bacterial wilt, moko, bugtok, *Ralstonia solanacearum*

BSD No. 11

TAXONOMIC CHARACTERIZATION OF A NOVEL RHIZOBIAL STRAIN ISOLATED FROM ROOT NODULES OF ENTADA PHASEOLOIDES

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Rhizobia are symbiotic bacteria capable of eliciting root and stem nodules on leguminous plants, where they reduce atmospheric nitrogen to ammonia to the benefit of the plants. Initial studies on rhizobial diversity in Okinawa, Japan revealed that MAFF 210191 isolated from root nodules of woody legume *Entada phaseoloides* exhibited phylogenetic characteristic distinct from other rhizobia. However, phenotypic characteristics of this isolate have not been described. Thus, this study was done to characterize and examine the taxonomic position of MAFF 210191 using polyphasic approach. Results showed that MAFF 210191 belong to the slow-growing group of rhizobia, displaying biochemical, physiological and chemotaxonomic characteristics quite different from the other rhizobia. Phylogenetic analysis based on 16S rDNA sequences showed that this isolate formed a separate node far from other root-nodulating bacteria. Analysis of its *nodA* gene (encodes an acyltransferase involved in nodulation) sequences showed a high homology with that of *Rhizobium tropici* CFN 299. These results suggest that MAFF 210191 occupies a unique phylogenetic position distinct from other rhizobia, and probably a new genus of nodule-forming bacteria. In light of these, characterization of symbionts of yet unexplored legumes such as *E. phaseoloides* reveals additional rhizobial species. Such undertakings may significantly contribute to the understanding of the origin and evolution of the rhizobium-legume symbiosis, and open new perspectives for environmental and agricultural applications.

Keywords: polyphasic taxonomy, rhizobia, root nodules, 16S rRNA, *Entada phaseoloides*

BSD No. 12

GENOME IDENTIFICATION OF SELECTED TABLE AND COOKING-TYPE BANANA (*Musa* Sp.) CULTIVARS IN THE PHILIPPINES THROUGH ISOZYME ANALYSIS

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Isozyme profiles of twenty one table-type and six xooking-type banana cultivars were studied. The different isozymes include malate dehydrogenase (MDH), 6 phosphoglucodehydrogenase (PGD), phosphoglucoisomerase (PGI), and Phosphoglucomutase (PGM). Different banding patterns were observed. Isozyme patterns in the different cultivars to determine its genome (19) were compared with eight (8) newly collected cultivars to determine its genome identity. The genomes of the five table-type cultivars were identified. Based on MDH and PGD, the genome of Latundan Puti is AAB, the genome of Manifun and Magipod is AAA. The four isozymes were not useful in identifying the genomes of the three cooking-type cultivars namely, Balatay, Bataan and Dumanese. Unweighted Pair Group Method using Averages Cluster Analysis confirms the correct genome of the five table-type cultivars.

Keywords: table-type bananas, cooking-type banans, isozymes, malate dehydrogenase, phosphogluconate dehydrogenase, phosphogluvoisomerase, phosphoglocomutas

BSD No. 13a

SCREENING OF DNA POLYMERASE FROM LOCAL HYPERTHERMOPHILES

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DNA polymerase is the enzyme involved in amplifying segments of DNA in an in-vitro technique termed as Polymerase Chain Reaction or PCR. Because of its speed and specificity, the PCR technique has found numerous applications in molecular biology research, epidemiological and forensic studies, disease diagnosis, and pathogen detection. This study aimed to screen the DNA polymerase in local hyperthermophiles.

A total of 150 isolates from hotsprings and mudsprings were obtained by streaking onto three different media, namely: modified modified artificial sea water (ASW) medium, nutrient broth and DSM medium. Twenty isolates have been purified but only 10 remained in stable conditions using the modified *Thermus* medium. Most of these isolates were found to grow at 85 °C.

Detection of a homologous DNA polymerase gene from the genomic DNA templates were done using *Thermus aquaticus* (*Taq*) polymerase gene (TP) primers. The positive control which was the *Taq* pol recombinant clone, p*Taq*, produced the expected 2.5 kb fragment. Isolates 62d, 62c, 60b2 and 62h2 each produced an amplified product 1.2 kb in size. Isolate 571aa and 10e had approximately 1.5 kb product whereas the Mudspring isolates had < 1 kb as its major amplified product.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein lysates showed that only three out of the eleven protein precipitate of the thermophilic bacterial isolates exhibited the protein band of approximately 90 kd. These initial results gave us the information on which isolate to choose for the purification of the protein and cloning of the DNA polymerase gene in *E. coli*.

Keywords: DNA polymerase, thermophilic bacteria, PCR-based screening

BSD No. 13b

NEUTRAL PROTEASES OF THERMOPHILIC *Bacillus* sp. ISOLATED FROM THE MUDSPRINGS, MOUNT MAKILING, PHILIPPINES

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Six thermophilic *Bacillus* sp. (3SM-23, 4MM-2, 4MM-22, 5-SM-6, 7MM-8 and 7MM-16) from the Mudsprings, Mount Makiling, Los Baños, Laguna, were selected and screened for neutral protease production using modified soybean cake extract broth. Crude enzymes produced by the *Bacillus* sp. were assayed using 0.6% casein and results obtained showed that three isolates, 3SM-23, 7MM-8 and 7MM-16, have high neutral protease activity (NPU) of 183.9, 128.7 and 154.1, respectively. Crude neutral proteases (CNP) from these isolates have maximum proteolytic activity at pH 7 and temperature of 55°C for 3SM-23 and 7MM-16, and at 40°C for 7MM-8. CNP were stable over a pH range of pH 4 to 7 for 3SM-23, 4 to 8 for 7MM-8 and 5 to 7 for 7MM-16. On one hand, the enzymes were stable at temperatures 20 - 60°C for 3SM-23, 20 -50°C for7MM-8 and 20 - 55°C for 7MM-16.

Keywords: Neutral protease, *Bacillus* sp., thermophilic bacteria

BSD No. 14

PREDICTION OF POTENTIAL CYTOTOXIC LYMPHOCYTE (CTL) EPITOPES IN MALARIA ANTIGENS USING COMPUTATIONAL TOOLS

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Induction of cytotoxic T cell (CTL) response against *Plasmodium falciparum* through the use of antigenic determinants as vaccines can greatly

reduce malario infections worldwide. CTL responses have been theorized to be important in controlling liver stage malaria infection. Identification of T cell epitopes through experimentation alone is laborious and expensive, creating the need for computational tolls that can decrease the number of candidate epitopes to make T cell epitope identification faster. Four proteins expressed by the parasitic during this stage, namely liver stage antigen-1 (LSA-1), liver stage antigen-3 (LSA-3), merozoite surface protein-1 (MSP-1), and thrombospondin-related anonymous protein (TRAP), were searched for candidate T cell epitopes through the use of prediction servers available online. The following software were used in the study: ProPred1. RANKPEP, SYFPEITHI, PREDEP, and an unnamed prediction tool. For the HLA class I alleles known to be prevalent in the Philippines, 40-180 epitopes from each of the four proteins were predicted by the methods used. Some of these epitopes have already been experimentally tested by others. Analysis of promiscuity was also done to identify epitopes that can potentially be presented by multiple alleles of MHC class I molecules from different loci. Seven epitopes from TRAP appear to be promiscuous, while none were found for LSA-1. Selected T cell epitopes among the hundreds predicted can then be tested in *in vitro* binding experiments to confirm their specificity.

Keywords: malaria, T cell epitope, HLA class I, prediction, LSA-I, LSA-3, MSP-1 and TRAP

BSD No. 15

CONSTRUCTION OF SHUTTLE PLASMIDS WHICH CAN BE EFFICIENTLY MOBILIZED FROM *Escherichia coli* INTO THE CHROMATICALLY ADAPTING CYANOBACTERIUM, *Fremyella diplosiphon*

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In some strains of cyanobacteria the composition of the light-harvesting antennae is determined by the color of available light. The mechanisms of this chromatic adaptation involves the regulation of gene expression by red and green

light and has been most studied in *Fremyella diplosiphon (Calothrix* sp. FCC7601) a filamentous cyanobacterium for which there has been no reported means of genetic manipulation. We have constructed shuttle plasmids which can be efficiently mobilized by RP4 from *Escherichia coll* into *Fremyella diplosiphon* and which can be recovered from transconjugant *F. diplosiphon* and turned to *E. coli* by transformation. The ability of these plasmids to replicate in *F. diplosiphon* is conferred by an 8.0-kb DNA fragment isolated from pFDA, a plasmid native to *F. diplosiphon*. To create these shuttle plasmids from *oriV* and *bom* from pBR322, *cat* from pACYC184 and *aphA* from pACYC177. pJCF22 lacks sites for the restriction enzymes *Fdi*I and II. Transconjugants *F. diplosiphon* containing shuttle plasmid pJCF62 are resistant to chloramphenicol and highly resistant to the aminoglycosides, G418 and neomycin. When *aadA* from the omega interposoa was incorporated into a shuttle plasmid transconjugant *F. diplosiphon* could also be selected with streptomycin or spectinomycin. In *F. diplosiphon* shuttle plasmid pJCF62 replicates with a minimum copy number of seven. The *oriV* for replication in *F. diplosiphon* was localized to a 2.8-kb region within the cyanobacterial part of pJCF62. The presence on a shuttle plasmid of a single recognition site for *Fdi*I reduced the efficiency of mobilization into *F. diplosiphon* by 5-to 10-fold Restriction at this site was prevented when the *E. coli* donor strain in the mating contained the enzyme *Eco*4711 methylase.

Keywords: cyanobacteria, chromatic adaptation, *Fremyella diplosiphon*, shuttle plasmids, *Escherichia coli*

BSD No. 16

GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC TO THE CD33 CELL SURFACE ANTIGEN OF LEUKEMIC MYELOID HL-60 CELLS

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The CD33 antigen is a 67 kD cell surface sialoadhesin molecule that is primarily expressed on normal progenitor monocytic and nature myeloid

hematopooietic cells, but not in non-myelomonocytic nor in non-hematopoietic cells. Because CD33 serves as a marker for myeloid progenitor cells and most leukemic myeloid cells, monoclonal antibodies (mAbs) that are reactive with this CD33 glycoprotein cell surface antigen serve as effective agents in the immunotherapy of acute myeloid leukemia (AML). In this study, mAbs specific to CD33 expressed by HL-60 cells were generated and characterized. M195 hybridoma cells, producing murine anti-CD33 IgG_{2a} mAbs, were grown in vivo and injected into primed BALB/c mice for propagation in ascitic fluid. The anti-CD33 mAbs were then purified from the extracted ascitic fluid by fast performance liquid chromatography (FPLC) on Protein G columns. Enzyme-linked Immunosorbent Assay (ELISA) was performed to characterize the binding affinity of the purified anti-CD33 mAbs to CD33-positive human leukemic myeloid HL-60 cells. The purity and the molecular weight of the anti-CD33 mAbs were subsequently determined by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE). The generated, characterized, and purified anti-CD33 mAbs are useful in the production of cytotoxic drug-antibody bioconjugates for drug therapy and ex-vivo purging of bone marrow prior to autologous transplantation. Ultimately, the engineering of these anti-CD33 bioconjugates by pepsin digestion and mild reduction into F(ab') fragments optimizes the specificity to leukemic myeloid cells displaying AML, and decreases immunogenicity to normal myeloid cells.

Keywords: CD33, HL-60, M195, monoclonal antibody, hybridoma, acute myeloid leukemia

BSD No. 17

TAXONOMIC REASSESSMENT OF LOCAL ANTIBIOTIC- AND ENZYME-PRODUCING *Bacillus* ISOLATES BASED ON PHENOTYPIC CHARACTERISTICS

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The taxonomy of fifty-four locally isolated antibiotic- and enzyme producing *Bacillus* isolates deposited at the Philippine National Collection of Micro-

organisms (PNCM)-BIOTECH were reassessed in this study since they were only partially identified prior to deposition in the culture collection. Results from thirty-nine phenotypic tests were analyzed using simple matching coefficient to construct a dendrogram. The dendrogram showed twelve clusters discerned at 80% similarity level - cluster I consisted of *B. cereus* and related species, clusters II to V included the *B. subtilis* and related species, and clusters VI to XII appeared to be single-member clusters. Molecular analysis using 16S rRNA-specific PCR primers further differentiated the isolates belonging to cluster I (*B. cereus* group) from those of clusters II to V (*B. subtilis* group). Based on these analyses, forty-three isolates of *Bacillus* maintained their original identities, while five isolates were named at the species level. Nine isolates were misclassified prior to deposition, and were re-identified and renamed.

Keywords: *Bacillus*, taxonomy, phenotype, cluster analysis

BSD No. 18

DENITRIFYING BACTERIA IN THE SEDIMENT OF CAGED AND UNCAGED SITES IN LAKE TAAL

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Nitrate loss through microbiological denitrification is one way of bioremediation of nitrate-rich environments. The total counts of denitrifying bacteria in the sediment of caged and uncaged sites in Lake Taal were determined using the most probable number (MPN) technique with 10x diluted nutrient broth + KNO_3 (NBN) as medium. Phenotypic and biochemical tests using established procedures and the API 20 NE system were done on pure cultures isolated from highly diluted MPN cultures showing positive nitrate reduction. Results show that the caged site had MPN of 1.8×10^4 per g dry wt sediment while the uncaged site had 2.4×10^1. The % of denitrifying bacteria of total aerobic heterotrophic bacteria in the caged site was 0.08%, in the uncaged site 0.0005%. Except for one , the isolates were gram-negative rods, motile, and oxidative. The predominant denitrifyers in the caged sites were identified as *Pseudomonas aeruginosa* and

Aeromonas hydrophila; in the uncaged site, *Chryseomonas luteola*. These bacteria must play an important role in the sites by reducing the nitrate level in the environment.

Keywords: Denitrifying bacteria, Lake Taal, *Pseudomonas aeruginosa*, *Aeromona hydrophila*, *Chryseomonas luteola*

BSD No. 19

TAXONOMY, DISTRIBUTION AND TEMPORAL CHANGES IN THE ABUNDANCE OF PHYTOPLANKTON IN TAAL LAKE, BATANGAS

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Taal Lake, a volcanic lake and one of the known tourist spots in the Philippines has a significant contribution to the country's economy being also one of the sources of large amount of commercial fishes distributed to nearby provinces. The introduction of commercial fish cages and the river inputs have contributed a great deal of disturbance in the lake's ecosystem. To investigate the lake's water quality and phytoplankton composition, a study was conducted from October 1999 to July 2000. Parameters such Ammonium-Nitrogen (NH_4-N), Nitrate-Nitrogen (NO_3-N), soluble Phosphate ($P_{s.s}$) and Total Phosphorous (P_T), temperature, light penetration, pH, total dissolved solids (TDS) and conductivity were measured from the three sampling sites. Qualitative and quantitative composition of the phytoplankton from the identified sampling stations were also studied including chlorophyll *a* (chl *a*) concentrations.

Significant increase in NH_4-N was observed from June and July, 2000. Significant increase in P_T, P_s and NO_3-N were also observed during the dry season from the monthly collections conducted. Though there was a significant increase in the nutrient hold of the lake, there was no significant correlation between the nutrients and the chl *a* content of the three collection sites in the

lake. On the other hand, TDS ($p<0.05$), pH, and temperature ($p<0.001$) showed positive correlation with chl a concentration. There was no significant difference between depth observed among the nutrients and other physico-chemical factors. No significant increase in the monthly chl *a* collected but significantly high amount of chl *a* was observed from 2.5 m and 5 m depth. *Ceratium*, centric diatoms, *Aulacoseira* and *Merismopedia* showed significantly high individual cell count among the rest of the collection months considered for counting which also holds true to chl a concentration. The four significantly dominant genera mentioned above also showed positive correlation with TDS, pH, temperature, NO_3-N and NH_4-N generated from the Cannonical Corespondence Analysis (CCA).

Keywords : Taal Lake, phytoplankton, nutrients, water quality

BSD No. 20a

PHILIPPINE WILD MACROFUNGI WITH COMMERCIAL POTENTIAL: CONTINUING SEARCH AND CHALLENGE

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The Philippines as a tropical country is endowed with very rich and diverse flora and fauna which are still under utilized and undiscovered fro their economic potential. Wild edible macrofungi for instance are naturally found growing on forest litters, fallen logs and leaf debris. These microfungi are usually being ignored due tolack of technical information about their edibility by the local folks and due to the unavailability of production technology. In our attempt to domesticate these wild edible species, our group has initiated the collection, identification and rescue of their mycelia. We have been successful in the development of production technology for *Collybia reinakeana*, a virtually unknown edible species of wild mushrooms that usually inhabits the forest floor of Puncan, Carranglan, Nueva Ecija. It can be grown on composed rice straw - based substrates having a pH of 6.0 with >70% moisture content at

an optimum temperature of 30oC and 85% relative humidity. Four edible species of macrofungi which are known by the Aetas of Mt. Nagpale, Abucay, Bataan were also collected and rescued. These are *Schizophyllum commune*, *Ganoderma lucidium*, *Auricularia* sp. and *Mycena* sp. These mushrooms grow best at a pH range of 5.5-6.0 in a coconut water medium.

Keywords: *Collybia reinakeana*, *Ganoderma lucidum*, *Schizophyllum commune*, wild edible mushroom

BSD No. 20b

APPLICATION OF PURE AND MIXED POPULATIONS OF EFFLUENT-DERIVED MERCURY-RESISTANT BACTERIA IN BIOREMEDIATION

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Toxic heavy metal contamination of the environment is one of the most destructive forms of chemical pollution. Reports of fish kills and illnesses in certain fishing villages in Iligan City suspected of being caused by mercury poisoning serve as an impetus for identifying and ascertaining point sources of the toxic pollutant(s) and for developing strategies for remediation.

Our study focuses on the use of biosorptive properties of microorganisms for bioremediation applications. Four five hundred milliliter of effluent water and four 500-gram sediment samples were obtained from an effluent outlet conveying wastewater from two chemical plants in Iligan City. Serial dilutions were prepared and 0.1 ml portions were pour-plated using MS agar medium containing from 10 to 40 ppm of $HgCl_2$ for screening. The plates were then incubated at 37° for 48 hours. Biosorptive efficiency for mercury was performed in MS broth with 20 ppm $HgCl_2$. Mercury concentrations of the broth and cell pellet were measured using atomic absorption spectrophotometry (AAS) after 0, 24, 72 and 120 hours.

Isolate 4D showed a relatively high biosorptive efficiency of 78.56% after 72 hours when used as a single population. Isolates 2D and 1S exhibited increased biosorptive efficiency when used as a mixed population with a 1:1 ratio exceeding 90% after 72 hours.

We have therefore isolated bacterial strains that could be excellent candidates for bioremediation in heavy metal-contaminated environments. Further isolate characterization and design of cell immobilization systems for use in industrial effluent outlets are on-going endeavors in our laboratory.

Keywords. bioremediation, biosorptive efficiency, atomic absorption spectrophotometry, cell immobilization

BSD No. 21

DETECTION OF MICROCYSTIN FROM CRUDE EXTRACTS OF *Microcystis* Sp. COLLECTED FROM LAGUNA DE BAY AND ITS EFFECT ON TILAPIA (*Oreochromis niloticus* L.) FINGERLINGS

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Laguna de Bay is extensively being used for aquaculture and is considered an important source of livelihood for families living along its coastline. At present, the lake faces problems such as runoff, and siltation. Moreover, dumping of domestic and industrial wastes contributes to the formation of blooms of cyanobacteria which may be capable of producing toxins in the lake.

The most common cyanobacterial toxin encountered in fresh water is the cyclic heptapeptide microcystin which inhibits protein phosphatase type 1 (PP1) and type 2A (PP2A), which can both be found only in eukaryotic organisms. Microcystin is also a hepatotoxin which causes severe hepatic haemorrhage and possibly liver cancer.

In this study, crude extracts of a Microcystis sp.were assayed for the presence of microcystin. Moreover, the pathologic effect of the cyanobacteria on tilapia fingerlings (Oreochromis niloticus L.) was also determined.

Masses of the cyanobacterium were collected and extracted using absolute methanol. Microcystin was then detected by intraperitoneal injection on ICR strain laboratory mice. The mice showed classical symptoms of microcystin in-

toxication which indicated the presence of the toxin. The presence of microcystin was confirmed by thin layer chromatography (TLC) with the elution of toxic spots having retardation factor (RF) values of 0.660, 0.701, and 0.7045 which are close to literature values.

Meanwhile, three to four month-old tilapia fingerlings were fed with fresh cells of the Microcystis sp. for seven days. Deaths were observed after the 5th and 3rd day of exposure to concentrations of 1g and 2g cyanobacterial cells per liter, respectively. Hotelling's Trace statistical analysis showed that the treatments had a significant effect on the accumulated deaths of the fingerlings. Histopathological examinations revealed pronounced effects on the liver with the hepatocytes becoming enlarged and vacuolated, with the nuclei displaced at the periphery, and with lesions on all hepatic lobules.

Keywords: microcystin, Microcystis sp., tilapia, Oreochromis niloticus L., cyanobacteria

BSD No. 22

GLUTATHIONE-S-TRANSFERASE PROFILE OF CAGED-CULTURED TILAPIA IN LAKE TAAL

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Tilapia farming has emerged as a major global industry for food production in the 20th century and cage culture has been one of the rearing methods extensively practiced at present. This study presents the glutathione S-transferase (GST) profile of tilapia cultured in Leviste at Lake Taal, Batangas and relates it with the water quality conditions. GST is a detoxification enzyme found in the liver and levels of it are increased during stress.

The GST activity of tilapia liver samples from Leviste was determined for the months of October, November 2001, and January 2002. Fish were acclimatized in the laboratory for 24 hours. Liver samples were dissected, homogenized in cold phosphate buffer. Homogenate was ultracentrifuged at approximately 100,000g at 4° C. Supernatant containing the cytosolic GST was stored under ultra-low

refrigeration for subsequent analysis. Phosphate buffer and 2 mM glutathione were added to diluted samples. The spectrophotometer was set at 340nm and the enzyme activity was measured as the absorbance change/minute. GST levels steadily increase from October to January, that is, 0.04, 0.09, and 0.12 dA/min, respectively . The increase in GST values is correlated with the steady decline of water temperature in the succeeding months October to January (29.6, 28.4, 26.6 ° C). As to dissolved oxygen and pH , lowest values of 2.97 mg/l and 7.9, respectively coincided with the GST peak of 0.12. This suggests that fish in cultured cages sampled in January seemed to be the most stressed ones.

Keywords: glutathione -S-transferase, liver, tilapia, culture cages, Lake Taal, Batangas

BSD No. 23

ERYTHROCYTE VALUES OF CAGED NILE TILAPIA IN LAKE TAAL INFECTED WITH GILL FLUKES

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Oreochromis niloticus or Nile Tilapia is the predominant fish cultured in Lake Taal. Erythrocyte values and metazoan parasites of caged Nile Tilapia from the lake were studied from August 2001 to May 2002. Seven to 20 specimens were collected monthly for eight months from two sites in the lake. Monthly total erythrocyte counts and % hematocrit of Site 1 (Quiling) fish ranged from 1.41 to 1.97 x 10^6 mm^{-3} and 20.8 to 37.0 %, respectively. Site 2 (Leviste) values ranged from 1.54 to 2.14 x 10^6 mm^{-3} and 23.5 to 38.2%, respectively. *Cichlidogyrus* spp. were recovered from the gills of most fish from both sites. Monthly prevalences and mean intensities of infection in Site 1 fish ranged from 77.8 to 100% and 7.7 to 46.8 parasites, respectively. In Site 2, values ranged from 86.7 to 100% and 10.2 to 134.7 parasites, respectively. Red cell values of fish from the two sites were similar and fall within the range of normal values for teleosts. High prevalences of infection in samples were observed from both sites, the higher mean intensity values in Site 2 may reflect poorer cultural conditions in the site such as overcrowded cages.

Keywords: Nile Tilapia, Lake Taal, *Cichlidogyrus*, erythrocytes, hematocrit, gill parasites

BSD No. 24

IMPORTANT INSECT PESTS OF SELECTED LIVE ORNAMENTALS IN NURSERIES AND NATURAL STAND

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Live ornamental plants like *Mussaenda, Aglaonema* and palms are now becoming important crops with export potential. These crops can be grown as potted plants that comman higher price. The plants are used as indoor and outdoor decors and landscape. Little is known as to the occurrence of insect pests on these ornamentals.

Based on the survey conducted in ornamental nurseries and natural stand of the crops from most part of Luzon including Palawan, the Visayas (Tagbilaran City and Ubay Bohol; Cebu City and Mandaue City) and in Mindanao (Davao City), showed that mealybugs, *Pseudococcos* sp. and aphids, *Aphis gossypii* Glover, are the most serious insect pests of *Mussaenda*. Occasionally, tussok moth larvae, *Orgyia australis postica* Walker occur at a damaging level particularly during rainy months. The red spider mites, *Tetranychus* sp. is also serious in greenhouses during the summer months. On *Aglaonema*, mealybugs and scale insects are the more dominant insect pests collected while the bagworms occasionally occurred in high number causing considerable damage on Manila palms and Champaign palms.

Survey on the crop protection practices of crop nurseries showed that nursery owners are more concerned of plant diseases than insect pests. Whenever insect pests are observed, owners spray available insecticied blanket to all ornamentals grown in the nursery.

Keywords: insect pest, live ornamental, *Mussaenda, Aglaonema*, palms, mealybugs, aphids, tussock moth larva, red spider mites, bagworms.

BSD No. 25

GENETIC VARIATION IN NATURAL POPULATION OF HONEYBEES *Apis cerana* F. IN MT. MAKILING AND ALONG LAGUNA DE BAY AREAS USING ISOZYME ANALYSIS

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Starch gel electrophoresis of 45 nursed bees per feral colony of *A. cerana* F. from 3 localities in an upland area, Mt. Makiling and lowland areas along Laguna de Bay showed polymorphism for acid phosphatase-2 (Acph-2), alkaline phosphatase (Alph), esterases (Est) and malic enzyme (ME) and monomorphism for acid phosphatase-1 (Achp-1).

Among the 8 presumptive loci observed S (slow), M (moderate) and F (fast) isozymes were noted for Alph-2, Est-2 and ME; only S and F for Acph-2, Alph-1, Est-1 and Est-3, while only S for Acph-1. Only Acph-1 and Est-2 in the upland areas and Acph-1 and Acph-2 in the lowland areas showed a goodness of fit to the Hardy-Weinberg equilibrium.

Genotypes Acph-2 SF, Est-2 MF, Est-3 SF were observed only in Sta Cruz, Alph-2 MM in San Pablo and Anos, Est-1 SF exhibited in Forestry, Est-2 MM and ME MF were unique to Sta Cruz and Anos.

Localities within each area showed very high degree of genetic identity and did not vary much in terms of the types of alleles. The two areas had equal proportion of polymorphic loci (P) however greater average heterozygosity (H) was observed in Laguna de Bay populations. No significant difference in enzyme variability was observed between the pooled upland and lowland populations of Laguna based on P, H, average number of alleles per loci (A), genetic identity (I_N), genetic distance (D) and genotypic similarity (I_H).

Keywords: *Apis cerana* F., honeybee, isozyme, polymorphism, acid phosphatase, alkaline phosphatase, esterase, malic enzyme

BSD No. 26

SOME STICK AND LEAF INSECTS (PHASMATODEA) FROM MOUNT MAKILING, LAGUNA

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The stick and leaf insects (Phasmatodea) are among the most unique and important features of terrestrial arthropod biodiversity in the tropics. This preliminary study was conducted to gain initial knowledge on the phasmatodean fauna of Mount Makiling with a view to expand later to more extensive faunistic/ taxonomic studies of Philippine Phasmatodea. Four stick insects and two leaf insects are identified from specimens gathered in limited initial field work and as well as collections available at the Entomology Section of the UPLB Museum of Natural History. They are *Lonchodes mindanaense, L. nodulosus, Orthomeria pandora Pharnacia ponderosa, Phyllium sp. nr celebicum and Phyllium* sp. In addition, there are two undetermined stick insects belonging to the subfamily Platycraninae of Phasmatidae and Necrosciinae of Heteronemiidae. The nocturnal habit of these insects as well as the limited time and funds available have not favored more extensive collections and field work. However, despite the limitations, these initial results suggest that further and bigger studies on the Phasmatodea of Mount Makiling in particular and of the Philippines in general are worth pursuing and that more new species and new records await discovery.

Keywords: stick insects, leaf insects, Phasmatodea, Mount Makiling, terrestrial arthropod biodiversity

BSD No. 27

TAXONOMY OF PHILIPPINE LAC INSECTS (KERRIIDAE, COCCOIDEA, HEMIPTERA)

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Lac insects are sources of commercial shellac, the raw material for varnish and other industrial products. They are not very common in the Philippines, there being hitherto only two known species that are difficult to recognize based on available antiquated and inadequate descriptions and illustrations. This taxonomic study was therefore conducted to establish their validity as species and facilitate their identification. *Kerria (Chamberliniella) greeni* (Chamberlin) and *Paratachardina minuta* (Morrison) are hence redescribed from specimens loaned from American and British natural history museums. These two have not been recollected since they were described some 80 years ago. *K. greeni* was originally described from specimens collected on *Ficus ulmifolia* on Mount Makiling whereas *P. minuta* was originally described from leaves of *Mangifera indica* on Basilan Island and was listed as a 'pest' of mangoes.. A third species belonging to the genus *Paratachardina* was collected from *Ficus* sp. from Imugan, Sta. Fe, Nueva Vizcaya and is also described as new to science. Scientific illustrations and a key to facilitate identification of adult females are provided and their possible conservation status is also noted.

Keywords: lac insects, Hemiptera, Kerriidae, Coccoidea, *Kerria*, *Paratachardina*

BSD No. 28

Coniothyrium zuluense: FIRST REPORT ON LEAF AND STEM CANKER OF *Eucalyptus camaldulensis* IN THE PHILIPPINES

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This study attempted to isolate and identify the causal agent of a leaf and stem canker disease observed in *Eucalyptus camaldulensis*. Distinguishing morphological characteristics were investigated using a scanning electron microscope (SEM). A fungal organism consistently isolated from infected tissues as well as in pathogenicity testing was confirmed by SEM observations to be *Coilothyrium zulense* of Class Deuteromycetes (imperfect fungi), Order Sphaeropsidales. It can be cultured, producing pycnidia in artificial media. Symptoms initially appeared as measle-like spots, transforming into cankery appearance and eventually progressing into a necrotic lesion. Cracking of the main stem was observed in severely damaged tree. This is the first recorded fungal pathogen of *Eucalyptus camaldulensis* in the Philippines. It is one of the most serious threats to the species.

Keywords: leaf and stem canker, *Eucalyptus camaldulensis*, *Coilothyrium zulense*

BSD No. 29

THE ROLE OF PLANTS IN THE CULTURAL PRACTICES OF THE KALANGUYAS IN TINOC, IFUGAO, CORDILLERA REGION, PHILIPPINES

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The diversity of plant species in the Cordillera Region, the northernmost part of the Philippines is matched by an equally rich cultural diversity. However, both have not been systematically recorded and documented. This paper presents the relationship between humans and plants with the integration of the cultural practices regarding plant use among the *kalanguyas* in Tinoc, Ifugao Province.

The *kalanguyas*, who dominate the towering mountains of Tinoc, one of the Municipalities of Ifugao Province exhibit unique cultural practices. One aspect, which is the focus of this study, is plant utilization. The methods used were individual interviews, focused group discussions, field research and direct observation. Key informants were identified and participants included the senior citizens and mabakis (pagan priests). Taxonomic method was used in the collection and processing of useful plants. Voucher specimens served as materials for identification and validation.

There are 293 identified useful plant species in Tinoc, Ifugao. These are distributed to 96 families, which include the major plant groups: ferns, gymnosperms and angiosperms. Indigenous uses of plants among the *kalanguyas* are integrated into every facet of their daily lives. These uses ranges from the most basic use to a variety of uses such as food, clothing, shelter, adornment, cordage, dyes, toys, rituals, basketry, medicines, musical instruments, cosmetics, poisons, tools, transportation, weapons, soil and water conservation, ornamentals and many more. The list is endless. To this indigenous group, the forests served as their natural grocery store, the pharmacy and the hardware store. This indigenous knowledge of plant use by the *kalanguyas* has evolved for thousands of years. How they utilize plants can also help define the basic elements of their earlier culture.

The distribution of traditional knowledge on plant use indicated that the more aged segment of this indigenous group held much of the information hence there is a need to capture the indigenous knowledge before it is irretrievably lost to future generations.

Keywords: *kalanguyas*, indigenous practices, *mabaki*, Tinoc, Ifugao, ritual plants.

BSD No. 30

OUTSTANDING ENDEMIC HOYAS OF THE PHILIPPINES

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The Philippines possesses one of the richest and most diverse range of hoya species in the world. Hoya belongs to the Asclepiadaceae family. The plant is characterized by shiny waxy leaves, hence the common name "wax plant". Most of the hoya species have a climbing or viny habit, however some species are short and bushy. The flowers are so striking and beautiful resembling a star with sweet lemony fragrance at night.

In the Philippines, hoyas can be found all over the islands at all altitudes. To date there are about 51 species which have been identified. It is sad to note, however, that it is one of our most outstanding endemic ornamental plant which have been relegated to the background and was not given much interest and attention in terms of research and media mileage. Most of these species are now in private collections especially those avid hoya enthusiasts and rare ornamental collectors most of whom are foreigners. Their number is rapidly depleting and most of these rare hoyas have found their way to other countries. Thus, other countries which are less endowed in terms genetic resources continue to forge ahead and profit from a global hoya industry to the detriment of our national heritage and economy. Active measures like recollecting, preserving and utilizing these valuable germplasm have to be done now.

Keywords: hoya, endemic

BSD No. 31a

SCANNING ELECTRON MICROSCOPIC STUDIES OF THE LEAF EPIDERMAL FEATURES OF FOUR PHILIPPINE PLANTS AFFECTED BY CEMENT DUST POLLUTION

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The cement industry is considered one of the polluting enterprises in the Philippines. Dust from cement factory kilns, which is injurious to plant life, has a very high air pollution potential. The intensity of cement pollution in an area can be gauged via the degree of injury imparted on the morphological and physiological characteristics particularly the modifiable leaf epidermal characters.

In this study, leaves of four plant species viz., *Bougainvillea spectabilis* Willd., *Hibiscus rosa-sinensis* Linn., *Mangifera indica* Linn. and *Psidium guajava* Linn. from the vicinity of three cement factories (Central Cement and Hi-Cement in Bulacan; Rizal Cement in Rizal) and from presumably relatively unpolluted areas (Montalban, Rizal and Pulilan, Bulacan) were compared in terms of four features viz., (1) stomatal density, (2) stomatal size, (3) trichome density and (4) trichome length. This was done to determine their potentials as bioindicators of pollution. Quantitative measurements and statistical analysis using the MANOVA and Waller-Duncan K-ratio T-test showed significant differences between plants from cement factory sites and control sites. Stomatal density and trichome length significantly changed for *Psidium* while variation in stomatal density was highly significant in *Hibiscus*. There were no significant changes in the four features for Bougainvillea and Mangifera. Scanning electron microscopy showed that epicuticular wax is abundant in *Bougainvillea* and *Mangifera* leaves from cement factory areas in contrast to those from the control areas. Wax nearly completely occluded the chambers of most stomata of *Hibiscus* leaves from cement-polluted areas while it partially occluded the stomata of leaves from the control areas.

Keywords: bioindicators, cement, leaf, pollution, scanning electron microscopy, stomata

BSD No. 31b

MORPHO-ANATOMICAL RESPONSES OF *Blumea balsamifera* (L.)DC. (SAMBONG) TO DIFFERENT LIGHT ENVIRONMENTS

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Blumea balsamifera, an aromatic weed found in open and partly shaded areas was grown under three different light environments designated as low light (LL), medium light (ML) and high light (HL) to determine any morpho-anatomical variations in response to different light intensities for a period of two months. A fourth group was grown under greenhouse conditions as control. Plant height, stem thickness, specific leaf weight (SLW) and leaf surface area were noted. The anatomical features measured were total blade thickness, thickness of upper and lower epidermis, and mesophyll thickness. Results show that among the morphological attributes measured, only leaf surface area was significantly altered by exposure to the HL set-up. However, anatomical measurements show an increase in leaf thickness in both ML and HL set-ups as compared to LL and the control. The differences in leaf thickness was due to changes in the thickness of the mesophyll, brought about by concomitant increases of either the size of the cells or the number of mesophyll layers or both. The leaf anatomy was also characterized according to the size and shape of epidermal cells and the number of epidermal layers, presence of cuticle, location of stomata, the presence and type of crystals and trichomes, and the arrangement of vascular tissues in the midrib. The mesophyll of *B. balsamifera* is undifferentiated, although the layers comprising the mesophyll have distinct characteristics resembling palisade and spongy features. The results of this study will be useful in finding the optimum light conditions for large scale cultivation and effective management of this medicinal plant.

Keywords: crystals, trichomes, mesophyll, epidermis, vascular tissues

BSD No. 32a

IDENTIFICATION OF MOLECULAR MARKERS FOR MUNGBEAN (*Vigna radiata* (L) WILCZEK) BRUCHID (*Callosobruchuschinesis* L.) RESISTANCE BY RANDOM AMPLIFICATION OF BULKED GENOMIC DNA SAMPLES

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To identify Random Amplified Polymorphic DNA (RAPD) markers that are potentially linked to the bruchid resistance gene, 200 recombinant inbred lines from a cross between NM 92 (cultivated variety) and TC 1966 (a *V. radiata* var. sublobata accession resistant to bruchid) were bioassayed and the DNA were extracted. Results from the bioassayed revealed that 44 were highly resistant (seed damage= 0 %), while 38 were highly susceptible (seed damage< 80 %). The DNA of resistant individuals were bulked into two (R1and R2) and so were the DNA from susceptible individuals (S1 and S2). Both R1 and R2 were composed of 22 individuals while s1 and S2 were composed of 20 and 18 individuals, respectively. Parental DNA was screened using 240 Operon primers and 379 UBC primers for polymorphism. A total of 158 Operon primers and 204 UBC primers were able to distinguish polymorphism between the parents and these primers wee subsequently used in screening the bulk samples and the nearly isogenic lines (NILSs). From a total of 360 primers used in the screening of bulk samples, four primers (Operon T 16, Operon V 02, UBC 193 and UBC 313) produced fragments unique to the resistant samples including the resistant NIL. Thus, there is a possibility that these fragments are linked in cis with the bruchid resistance gene for they cosegregate.

Keywords: RAPD, bruchid resistance, *Vigna radiata*, genomic, DNA, molecular markers

BSD No. 32b

QUANTITATIVE AND QUALITATIVE ANALYSES OF CORN (*Zea mays* L. IPB VAR. 911) SEEDLINGS TISSUES EXPOSED TO LEAD NITRATE

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Responses of plants to toxic and essential substances are manifested in variuos levels of organization. Cellular aberrations may be present but are not reflected in the gross morpholy of plants. In this study, corn seeding grown in potted soil were subjected to various concentrations of lead nitrate [$Pb(NO_3)_2$]. After 21 days of treatment, harvested plant samples were processed using the Paraffin Microtechnique procedures. Prepared slides were analyzed using quantitative and qualitative anatomical parameters. Results revealed significant ($P<0.05$) enlargement of root pith, root cortex, stem ground parenchyma, stem vascular bundle, leaf vascular bundle and iameter of root metaxylem at the higher treatments [HT-2000 and 5000 mg kg-1 Pb(NO3)2]. A similar increase in the number of root metaxylem cell at 500 and 5000 mg kg-1 treatments was also noted. However, remarkable decreases in the number of root metaxylem cell, widths of root pith, root cortex at 100 mg kg-1 treatment and reduction of width of stem ground parencyma and stem vascular bundles at 500 mg kg-1 treatment were obtained, respectively. Photomicrographs of root cortical tissue exposed to HT showed disrupted cell walls. Likewise, there was an apparent damage of root metaxylems in the treated samples. Distortion of root cortex was observed at 2000 and 5000 mg kg-1 treatments, while aberration of stelar area was noticed at 500 mg kg-1 treatment. Results suggest that the nitrate counter ion brought the positive growth of tissues, however, the negative effects of Pb on cells and tissues were still apparent.

Keywords: corn, anatomy, nitrate, toxic heavy metal, Pb, analysis

BSD No. 33

LIGHT MICROSCOPY STUDIES ON THE PATTERN AND DISTRIBUTION OF CALCIUM OXALATE CRYSTALS IN *Azadirachta indica* L. (NEEM)

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Plants accumulate crystals of calcium oxalate and calcium carbonate in a variety of shapes, sizes, amounts, and locations. To gain insight into this phenomenon, the morphology and distribution of these crystals in mature leaves of *Azadirachta indica* L. (Neem) were studied using light microscopy. *Azadirachta indica* L. commonly known as Neem is considered as one of the most promising trees of the 21st century. It has great potential in the fields of pest management, in environment protection, in helping the control of diseases like malaria and AIDS, in combating desertification and deforestation, in reducing excessive global temperature, and even contribute to population control. Styloids and raphide crystals, some with peculiar forms, were observed in the epidermal cells of cleared leaves. Cross sections of the leaves also showed druse crystals located in the mesophyll layers. These three types of crystal forms found in different tissues indicate that calcium oxalate crystallization, a biomineralization process, does not occur at random but are always located in specific tissues.

Keywords: crystal, calcium oxalate, raphide, druse, neem, biomineralization

BSD No. 34

ESTROGENIC ACCTIVITY SELECTED PLANT CRUDE EXTRACTS ON THE MAMARY GLAND OF SARCUMA-INUCULATED FEMALE MICE

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This is a study on the estrogen receptor in mammary gland of sarcoma-treated ICR mice. The plants used have been identified to contain phytoestrogen – chemical which have similar structure to estrogen. They are implicated in the reduction of hormone-related cancers by competitively inhibiting

the binding of endrogenous cetrogens and xenoestrogens to receptor sites.

Female ICR mice, weighing 25-30 were given subcutaneous injection of 10 million sarcoma T 180 cells. The experimental group were treated with either garlic, ginger or onion crude extract, dose of 7mg/30 g body weight at three different schedules: 3 days before sarcoma injection, 3 days after sarcoma injection and simultaneous with sarcoma injection. Thereafter, each group was injected twice a week with crude until day 30 past sarcoma treatment.

Mammary glands were excised, fixed in 4% formalin and proceed by light microscopy. Immunohisto-chemical reactions were performed using mouse estrogen monoclonal antibodies. Qualification of estrogen receptor was evaluated at 400x following the protocol of Zava et. at., (1977)

Among the three extracts, garlic and ginger showed estrogen receptors.

Keywords: estrogen, sarcoma, mammary gland

BSD No. 35

EFFECTS OF *Carica papaya* L., *Hippobroma longiflora* (L.) G. DON., *Strophanthus cumingii* A. DC., AND *Vitex negundo* L. INVERTEBRATE NERVE, SKELETAL AND HEART MUSCLES

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Bioactive substances in plants, presumably, the defenses of the plants against their natural enemies affect animals in various ways. In this study, the effects of *Carica papaya* L. (papaya) *Hippobroma longiflora* (L.) G. Don (estrella), *Strophantus cumingii* A. DC., and *Vitex negundo* L. (lagundi) were tested on the toad heart *in situ*, toad sciatic nerve-gastrocnemius muscle preparation, and the isolated guinea pig atrium. The commercialized tablet of lagundi (Ascof) was also tested. The results of the experiment could be useful in the evaluation of the plants for their potential as source of drugs or in the case of *V. negundo* and *C. papaya* for their more effective management as herbal medicines. Ethanol

extracts of various parts of the plants at varying doses were applied either by immersion of the tissues in baths containing them or parenterally, as for the experiments with the toad heart *in situ*. The monitoring of responses to the treatments were done electronically. Notable results were the (1) 62% reduction of skeletal muscle activity by papaya seed (5%) with recovery to maximum 87% after washing, (2) significant neurotoxicity of estrella leaves and stems causing reduction of muscle activity, respectively, of 61.4% and 40.2%, (3) negative chronotropic effects of papaya seed (5% and 10%), (4) on the toad heart *in situ*, positive inotropic effects of S. cumingii young stems and flowers (5% and 10%) but negative inotropic effects at 20% and toxicity at 40%, (5) on the isolated guinea pig atrium, negative chronotropic and negative inotropic effects of lagundi leaves and tablet with initial positive inotropic effect only at low dose tablet, cardiotoxicity of 0.6% leaf and 0.4% flower extract, and (6) the inaction of lagundi on the muscarinic receptors of the heart. The data were analyzed by Student's T-test, analysis of variance, and Tukey's Test.

Keywords: *Carica papaya*, papaya, *Hippobroma longiflora*, estrella, *Strophantus cumingii*, *Vitex negundo*, lagundi, neurotoxin, neuromuscular toxin, chronotropic effect, inotropic effect

BSD No. 36

THE PROTECTIVE ACTION OF THE LEAF EXTRACTS OF *Mangifera indica* Linn., *Premna odorata* Blanco, AND *Psidium guajava* Linn. AGAINST ETHANOL-INDUCED LIVER LIPID PERODIXATION

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Studies have indicated association between health promoting properties of plant foods and medicines with active phytochemicals including antioxidants. In the present study, the antioxidant activities of the leaves of three plants namely

the mango (*Mangifera indica* var. *indica* Linn.), "alagao" (*Premna odorata* Blanco), and guava (*Psidium guajava* Linn.) were assessed. Ethanol extracts of young tender leaves, young mature leaves, and old mature leaves, were tested for their abilities to inhibit the lipid peroxidation that can happen in the liver of mice that were administered ethanol or that which can happen spontaneously. For a period of 7 days prior to the administration of alcohol (3g/kg) *per os* or distilled water, leaf extracts (1.5g/kg) were fed daily to the experimental animals. These tests were done alongside both positive and negative controls that were not giventhe plant extracts but treated respectively with ethanol and water on the eighth day following administration of water for 7 days. During the experiments, the animals had free access to pellet food and drink. Four hours after the last treatment, the mice were sacrificed, the livers excised and assayed for lipid peroxidation product malondialdehyde using the thiobarbituric acid reactive substances (TBARS) assay procedure. The results showed the significant inhibition of spontaneous lipid peroxidation by all types of leaves of *P. odorata* (28-40%) and the young tender leaves of *M. indica* (7%) and the significant inhibition of ethanol-induced liver lipid peroxidation of all types of leaves of *P. odorata* (102-124%), *M. indica* (93-111%), and *P. guajava* (97-111%). The lipid peroxidation in plant and alcohol-treated mice could go lower than negative controll levels. Maximum protection was offered by teh young tender leaves.

Keywords: *Mangifera indica*, mango, *Premna odorata*, alagao, *Psidium guajava*, guava antioxidant, lipid peroxidation, herbal medicines, TBARS assay