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BIOLOGICAL SCIENCES

WILDLIFE INVENTORY OF THE UNIVERSITY OF THE PHILIPPINES DILIMAN AND THE ATENEO DE MANILA UNIVERSITY CAMPUS, DILIMAN, QUEZON CITY, LUZON, PHILIPPINES

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An inventory of the terrestrial vertebrate species of wildlife in campuses of the University of the Philippines Diliman and the Ateneo de Manila University in Quezon City, Metro Manila, was conducted from October 1997 and August 1998. The UP Diliman Campus encompasses 493 hectares while the Ateneo de Manila University campus encompasses 83 hectares.

A total of 76 vertebrate species was recorded in the campuses of UP Diliman and Ateneo de Manila University. This diverse assemblage of wildlife in the study sites is comprised of 6 species of amphibians (1 endemic), 13 species of reptiles (2 endemic), 47 species of birds (7 endemic) and 10 species of mammals (1 endemic).

More than 61% of wildlife species found in the study areas were birds. Historical records of the assemblage of bird species in the UP Diliman area and its environs indicate that six species of birds used to be found in the study sites, are now no longer present

Keywords: biodiversity, UP Diliman and Ateneo de Manila, local extinction, endemic, wildlife

ZOOPLANKTON FROM LAKE TAAL

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Zooplankton were collected from January through August 1997 from three sites in Lake Taal. Water samples (12 L) were collected just below the surface and also near the bottom using a Van Dorn water sampler. Samples were filtered through a 53 μm plankton net and 5 mL formalin was added to each 10-mL collecting bottle.

Plankton collection was analyzed quantitatively using a Sedgewick Rafter counting chamber examined under a Carl Zeiss compound microscope. Triplicate counts were done per sample and the number of individuals per m^3 was calculated for each species. The species identified were one dinoflagellate and 22 rotifers. Also collected were copepods (larvae and adults) and cladocerans. The crustaceans e.g., *Lecane bulla* and *Hexarthra intermedia,* were found in almost all sites. Counts in thousands per m^3 were nauplii, 0-338.9; copepods, 0-138.9; cladocerans. 0-94.5; *B. forficula,* 0-61.1; *B. havanaensis,* 0-56-6; *L. bulla,* 0-208.3; and *H. intermedia,* 0-11.1. Peaks of abundance for the common species occurred around February and June.

Keywords: zooplankton, dinoflagellate, rorifers, copepods, cladocerans, crustaceans, nauplii, Lake Taal.

DISTRIBUTION AND ABUNDANCE OF ATYID SHRIMPS IN VOLCANO ISLAND SHORES, LAKE TAAL, BATANGAS

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The distribution and abundance of atyid shrimps (Family Atydae) were investigated in four sites along the shores of Taal Volcano Island, Batangas, Stations 1, 2 and 3 were on the northern part of the island, while Station 4 was on the midwestern part. Sampling was done for four months: July, October, and December 1995, and January 1996. Collection was done by towing a "pangkalap."

The predominant species collected was *Caridina gracilostris* de Man. Mean densities were highest in Station 4 with values ranging from 203/m^3 to 613/m^3

Mean densities in the other stations were usually <200/m^3. The abundant of atyids in station 4 may be due to its being sheltered from strong winds by the adjacent land mass in the west. This station also had the most abundance littoral submerged vegetation (*Vallisneria gigantea*). Vegetation was present but sparsely distributed in the other stations.

Keywords: atyid shrimps, *Caridina grascilostris, Vallisneria gigantea,* Lake Taal, Taal Volcano Island

LAKE TAAL'S ENDEMIC FRESHWATER SARDINE, *Sardinella tawilis:* SPECIATION IN PROGRESS OR GHOST OF ANCIENT POLYMORPHISM (INSIGHT FROM MOLECULAR DATA)

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Sardines belong to the genus Sardenella of the Family Clupeidae. In the Philippines, seven species under this genus have so far been described to share great morpho-anatomical similarities with one other. Of these seven, importance is being accorded to *S. tawilis* due to its endemicity only to Taal Lake and reports of its fast depletion. In order to delineate this freshwater sardine from marine sardines through identification of molecular markers that could also become basis for future management of *S. tawilis,* analysis of the sardines' mitochondrial DNA was conducted.

Three marine sardines, namely *S. albella, S. longiceps,* and *S. fimbriata,* that are geographically related to *S. tawilis,* were used. Two populations of *S. tawilis* (northern and southern) were studied, Dissected gonald and muscle tissues of the sardines were utilized in the extraction of mitochondrial DNA used in the amplification, cloning, and sequencing of the *cytb* and control region segments. Informative sequences were subjected to phylogenetic analysis using Neighbor-joining and Maximum-parsimony methods.

Results showed that the *cytb* of the sardines was 358 bp long which differed only in one nonsynonymous substitution that led to a change from isoleucine to valine. On the other hand, the presence of an 81-bp insert, a 35-bp tandem repeat present in up to eight copies and conserved sequence blocks (CBSs) in the control region sequences of *S. tawilis* and *S. albella* made them different from the rest of the

sardines. Phylogenetic analysis of the homologous sequences of the control region revealed that from the marine sardines investigated, *S. albella* was closest to *S. tawilis*. However, the possibility of finding closer one should not be dismissed since not all sardines were studied. Also, the two populations of *S. tawilis* were beginning to differentiate from each other as shown by the presence of population-restricted substitutions and by the single mutation in the *cytb*. The northern population was more homogeneous than the other probably due to physical barriers that may hinder gene flow or due to constant use of selective fishing gear in that area.

Overall, results of this study could serve as baseline information for future management of *S. tawilis*. Also, it opens interesting lines of study that would determine whether *S. albella* and *S. tawilis* are just similar to each other or are one and the same species that only became separated during the formation of Lake Taal.

Keywords: sardines, *S. tawilis*, *S. fimbriata*, *S. longiceps*, mitochondrial DNA, cytb, control region, conserved sequence blocks (CBS), phylogenetic analysis

A BIOMETRIC STUDY OF *Sardinella tawilis*, *S. fimbriata*, and *S. albella (Pisces:* Clupeidae)

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Five hundred specimens of *Sardinella tawilis*, *Sardinella fimbriata* and *Sardinella albella* were collected from Batangas Farmer's market in Quezon City. Thirty-seven biometric characteristics were subjected to factor analysis, discriminant analysis, and cluster analysis.

Results showed that among the 37 morphometric traits, the most consistent character was the fork length, while the most variable one was the length of the ventral fin. Among the meristic traits, the number of rays of the pectoral fin was the most consistent character, while the umber of rays of the anal fin was the most variable character.

Results of the factor analysis showed the presence of seven factors accounting for 77.65% of the variation among the species. Results of the discriminant analysis further revealed that only seven biometric characters sufficed to discriminate efficiently and accurately among the three species of Sardinella considered, namely; *S. tawilis*, *S. fimbriata* and *S. albella*.

Cluster analysis, which was presented in a dendrogram, revealed that the three Philippine *Sardines* can be distinguished from each other biometrically.

Keywords: *Sardinella, biometric, discriminant analysis, factor analysis, dendrogram.*

FISH COMPOSITION OF THE PANSIPIT RIVER: A COMPARISON WITH THE FIRST REPORT MADE 70 YEARS EARLIER

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Pansipit River is a 10-km single stream channel that serves primarily as an outlet of Lake Taal into Balayan Bay of the South China Sea and flows through the municipalities of Agoncillo, San Nicolas, Lemery and Taal. Commercially-important fish migrate into Lake Taal from Balayan Bay through this river. This study was conducted to determine: (1) the ichthyofaunal species composition of Pansipit River including Palanas River; (2) the abundance and seasonality of the important species under study; and (3) if the fish populations exhibit specific site/habitat preference or longitudinal distribution pattern.

Five sampling sites were established: four were along the Pansipit River (upstream, midstream 1, midstream 2 and downstream), and one in Palanas River, a diverging branch of Pansipit that also empties into Balayan Bay approximately 3 km from the mouth of Pansipit River. Monthly beach seine from February through December 1998 within the Pansipit and Palanas Rivers produced a total 59 species belonging to 37 families. Of the 59 species, 21 were caught exclusively in Pansipit, 26 in Palanas, while 12 were present in both rivers. Pansipit River supports a total of 33 species, 65% of which are migratory. Palanas River, on the other hand, supports a total of 38 species, of these 68% are migratory. Present species composition were evaluated with reference to earlier studies from the past seven years.

Pansipit River serves not only as a transport channel for fish, the villagers use the river for fishing, bathing, and laundering. Fish cages and fish corrals at the Pansipit River have also been erected in some parts of the river. The possible implications of human activities on the ichthyofauna of the river is discussed.

Keywords: fish, taxonomy, migratory, seasonality, distribution, Pansipit River, water quality, environmental pressures, biodiversity, conservation management.

SUBIC BAY FOREST RIVERS: WHAT MOLLUSKS DO THEY SUPPORT?

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Subic Bay is the former base of the U.S. Naval forces in the Philippines. The forest area of the base is approximately 18,000 ha. In 1992, the U.S. Naval forces left and this area was converted into an industrial and eco-tourism zone. The return of the base to the Philippine government paved the way for study of its biota – an activity not possible before due to security restrictions.

Quantitative analysis was conducted at the six major rivers within the Subic Bay Forest from November 1997 to July 1998. Each river system was divided into three sub-sites. Sampling per sub-site was conducted within the 100x100 meter quadrant. Specimens collected were identified based on Jutting (1956), Pace (1973) and Reeve (1860).

The diversity of the freshwater mollusks of the Subic Bay Forest Reserve was limited to two groups only: the Family Neritidae and Thiaridae which includes twenty gastropod species. this might have been possible consequences of the previous eruption of Mount Pinatubo. The species belonging to Family Neritidae are: *Clithon corona, Neritina coromandeliana, N. waigiensis, N. pulligera, Navicella barbonica, Septaria tesellata (S. lineata), S. porcellana,* and *Septaria sp.* The species belonging to Family Thiaridae are: *Thiara (Tarebia) granifera, T. scabra, T. winteri, Thiara* sp. 1, *Thiara* sp. 2, Melanoides asperata, *M. canilis, M. conchilidum, M. costata, M. plicaria, M. uniformis,* and *Sermyla riqueti.* Of the six river systems, Triboa River harbors the most number of species (16/20) followed by Ilanin River (9/20). Only two species were collected from Bayani River. Of the 20 gastropod species, M. asperata was found to be present in all the six rivers. This is followed by *Thiara granifera* (4/6). Nine out of the 20 species (9/20) were present each only in one river.

Melanids and *Neritina pulligera* serve as food to the native people who live within the Subic Bay Forest Reserve. These are commonly cooked with coconut. They also sell these Melanids outside Subic for 10 to 15 pesos per can.

Keywords: Subic Bay Forest Reserve, U.S. Naval Forces, biota, quantitative analysis, biodiversity conservation, Mount Pinatubo eruption, Neritidae, Thiaridae, Melanid

DIVERSITY OF MANGROVE VASCULAR FLORA AND ASSOCIATED MACROFAUNA IN MANGAL COMMUNITIES OF CATANDUANES

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The mangal communities of Catanduanes have not been the subject of various studies on community structure and function. Only fragmental studies have been made in the past. This paper fills in the gaps on the paucity of relevant information about diversity of mangrove flora and fauna in the island province. A taxonomic listing of vascular flora, icthyofauna and molluscan fauna, as well as preliminary data on their occurrence and distribution; species richness; and abundance of the major categories are included in this report.

Keywords: mangrove, macrofauna, mangal community, Catanduanes

OVARIAN CHANGES IN *Apongon themalis Cuvier* IN RELATION TO SPAWNING

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The ovarian changes in *Apogon thermalis* Cuvier from Lake Taal, Batangas were observed from May to January 1997, and from January to June 1998. Mean oocyte diameter and mean ganadosomatic index peaked twice – first in July and another in March. Histological studies of strained parafin section showed various stages of oocyte development: perinucleolar, yolk-vesicle, yolk granule and mature stages. Oocytes at the mature stage were observed in months when the mean ganadosomatic index and oocyte diameters were greatest. Above results indicate the *Apogon thermalis* spawn twice a year with the spawning season occuring between July and August, and between March and April.

Keywords: ovary, *Apogon thermalis,* oocyte, gonadosomatic index, spawning, histology, Lake Taal

PRELIMINARY ANALYSIS OF THE GENETIC STRUCTURE OF GIANT CLAM *(Tridacna crocea)* POPULATIONS FROM NORTHERN PALAWAN AND THE KALAYAAN ISLANDS GROUP (KIG)

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The South China Sea houses one of the most diverse assemblage of marine organisms in the world. Investigations of the genetic variability of selected reef organisms from different shoal and shelf reef systems in the South China Sea provides a basis for establishing affinities of reef-associated organisms within the South China Sea and among the bordering continental reefs. However, information on the genetic structuring of tropical marine organisms, particularly those from local reefs, is currently inadequate. Allozyme variation at five polymorphic loci was examined in three populations of *T. crocea* (KIG, Pangaldauan island and El Nido) to investigate the genetic affinities of the populations in the shoal reefs of the KIG and reefs on the North Western Palawan Shelf. Genetic distance (Nei's D) among populations ranged from 0.005-0.028, and as expected, increased with geographical distance F_{ST} values (mean=0.046) suggest that there is no genetic structring between the populations surveyed. In addition, high N_{am} values (4-6) suggest a high level of mixing of giant clam populations for the neighboring reefs.

Keywords: *Tridacna crocea,* allozyme markers; population genetics; giant clams, Kalayaan

MOLECULAR EVOLUTION OF β-GLUCANASES IN CEREALS

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The diversity, relationship, and pattern of molecular evolution of 23 β-glucanases in cereals were analyzed. The cereal β-glucanase is a structurally diverse class of enzymes with an overall protein sequence similarity of 74%. The size of the mature peptide is typically around 308 amino acids long with an estimated molecular weight of 32 to 35 kDa existing in either acidic and basic forms. Phylogenetic analysis using distance, maximum likelihood, and parsimony methods showed four distinct branches in the gene trees, designated subfamilies A to D. The sequence similarity within subfamilies was 76% for subfamily A, 93% for subfamily B, 87% for subfamily C, and 62% for subfamily D. While subfamily A had β1,3-glucanase activity and subfamily B had β1,3; 1,4-glucanase activity, the catalytic activities of subfamilies C and D are unknown nor can be predicted from their low overall similarities (68% and 60%, respectively) to both subfamilies A and B. Based on sequence homology, an orhologous relationship may exist between six pairs of the β-glucanase genes. The cereal β-glucanase genes exhibited an extreme G+C bias (94.6%) at the codon wobble position. The gene family registered a fast but fairly uniform evolutionary rate, with an overall rate of nonsynonymous substitution $Ka=3.6\pm0.11 \times 10^{-9}$/site/year in which subfamily B evolved slower than subfamily A. Different pairs of genes either observed or violated the molecular clock hypothesis.

Keywords: gene orthology, molecular clock, gene family, β-glucanases, cereals, evolution.

DNA SEQUENCES OF THE MITOCHONDRIAL 16s rRNA and CYTOCHROME B GENE LOCI OF THE PHILIPPINE HANGING PARROT, *Loriculus philippensis*

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The focus of this research is the DNA-based characterization of Colasisi or Philippine Hanging Parrot (*Loriculus philippinenses*) which is endemic to the Philippines. Optimized conditions for DNA extraction and polymerase chain reaction amplification of the 16s rRNA and cytochrome to gene loci were obtained for the colasisi blood samples collected from Laguna and Negros. The amplification of only the 16s rRNA gene fragment was optimized for the Davao colasisi sample. The PCR products were purified and successfully cloned into puc 18 cloning vectors as confirmed by restriction anzyme analysis and DNA hybridization. Cloned genes were subjected to automated DNA sequencing producing DNA sequence data for one of our very own endemic avian species in the country. The sequence data was analyzed to determine relatedness and to study the correlation of the genetic data with the geographic distribution of these parrots in the Philippines. Moreover, this research is one of the very limited efforts in our country to use molecular biology techniques in generating data towards the establishment of a genetic conservation laboratory for endemic wildlife species in the land.

Keywords: endemic wildlife species, sequence homology, Philippine hanging parrot, PCR, cloning, hybridization, 16s rRNA, cytochrome b

MAPPING HOMOLOGS OF DEVELOPMENTAL GENES *ENGRAILED* AND *HUNCHBACK* IN THE RED FLOUR BEETLE, *Tribolium castaneum*

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We have identified the location of the homologs of two developmental genes in *Drosophila melanogaster, engrailed* and *hunchback,* in the genome of the red flour beetle *Tribolium castaneum.* Two goegraphically-separated strains of *T. castaneum.* MMS (with visible morphological mutations) males were mated with T/W (wild type) females and then F_1 female daughters were mated back to their male parent to generate the F_2 mapping population. Portions of *engrailed* and *hunchback* genes in the genomic DNA of the parents, F_1 daughter and F_2 individuals were amplified by PCR (polymerase chain reaction) using opposing primers that correspond to intronic regions of both genes.

Well-amplified PCR products were subjected to SSCP (single strand configuration polymorphism) analysis. Each F_2 individual in the mapping population was scored for the presence or absence of a specific band derived from the T/W female parent. Data obtained were added to an EXCEL file containing the raw data for all RAPD (randomly amplified polymorphic DNA) markers and were analyzed using JOINMAP. The method assigned the location of *engrailed* and *hunchback* in chromosomes 7 and 5, respectively.

Keywords: mapping *Tribolium castaneum,* hunchback, engrailed, PCR, beetle

TRANSIENT EXPRESSION OF SALT-TOLERANCE GENES IN CYANOBACTERIA

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Cyanobacteria or the blue green algae are photosynthetic prokaryotes, many of which are capable of nitrogen fixation. They are deemed important in agriculture because of their role in the maintenance of soil fertility in paddy fields. However, secondary salinization from irrigation has become an increasingly serious and costly problem. Because of the economic importance of cyanobacteria, much interest is currently devoted to studies on the mechanism of salt tolerance in this group of organisms.

The present work elucidated the current hypothesis that stress adaptation is due to the expression of salt-tolerance genes. In this study, proteins from the unicellular forms designated as Lin-20 and Bat-09 were extracted at 0 h (control), 30 min, 1 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, and 10 h after exposure to different concentrations of 240 mM and 360 mM NaCl. The protein profile after electrophoresis using SDS-PAGE of Anabaena Btg 01 at 8 h and 10 h revealed new protein bands. In the case of isolate Bat 8, new bands were found after 6 h and 8 h of treatments. The Lin 20 and Bat 09 isolates did not show any change in their protein profile. Results the earlier data of the group that the filamentous forms are more salt-tolerant than the unicellular ones. These results are also consistent with the reported mechanisms involved in cyanbacterial salt tolerance which imply modification(s) in the synthesis and/or activity of cell proteins to facilitate osmotic adaptation.

Keywords: cyanobacteria, blue green algae, salinization, salt-tolerance, electrophoresis, SDS-PAGE, protein profile, filamentous, unicellular forms, osmotic adaptation

SCREENING FOR ENZYME PRODUCTING BACTERIA USING POLYMERASE CHAIN REACTION AND HYBRIDIZATION

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Enzymes have almost an infinite number of applications and the search for commercially-viable enzyme product often begin with screening for promising bacterial isolates. Locally-isolated protease-producing bacteria which were identified to belong to *Bacillus* species in an earlier study were used in order to develop a DNA-based screening procedure to obtain protease-producing bacteria using polymerase chain reaction (PCR). Primers for PCR that could amplify different regions of a gene for a neutral protease and a region of the subtilisin gene in Bacillus were designed. These primers were used to amplify the genes from the three identified *Bacillus* isolates. PCR profiles were analyzed and the PCR products were purified, labelled, and used as probes for detecting the presence of homologous protease genes using DNA hybridization. The use of PCR and hybridization was useful in detecting bacterial isolates that possibly possess protease genes. Results also indicate that DNA hybridization could be a useful tool not only for detecting protease genes but also for localizing these genes within the bacterial genome. Moreover, a similar screening procedure could be developed for the detection and localization of other commercially-important enzymes.

Keywords: protease gene, PCR, DNA hybridization, Bacillus, localization, primers, extracellular enzymes, neutral protease, subtilisin

OPTIMIZATION OF CONDITIONS FOR *Agrobacterium tumefaciens*-MEDIATED TRANSFORMATION OF RICE

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Plant growth regulators (PGRs), genotype explant type, and tissue culture conditions are some parameters that affect *in vitro* response of rice. We evaluated the response of 6 inbreds, 13 new plant types (ntp), and 6 cytoplasmic male sterile lines

(CMS) for their ability to produce embryogenic calli. Phenylacetic acid (10 mg/L) and 20 mg/L 2,4-Dichlrophenoxyacetic acid (2,4-D) were added to the culture medium as PGR. Spikelets of young inflorescence and mature seed embryos were used as explants to *Agrobacterium tumefaciens*-mediated transformation and incubated in the dark under light conditions.

PAA induced more embryogenic calli formation and plant regeneration to young infloresence (84.15%) than mature seed embryos (45.75%) even during light incubation.

Hygromycin-resistant calli conducted in light was more effective than in the dark conditions. Also, late selection with hygromycin gave a much higher percentage of Hyg^n calli (54.90%) and plants (14.22%) either in the light and dark conditions as compared to selection after Agro infection which ws 48.45% and 0%, respectively. Regenerated LX278 and LX286 plants were placed in the greenhouse. Molecular analysis will be conducted on these regenerants.

Keywords: genetic engineering, rice, crop improvement, binary vectors, *Agrobacterium tumefaciens*

EFFECTS OF TIME AND ENVIRONMENTAL CONDITIONS ON STR PRIMER AMPLIFICATION OF DNA EXTRACTED FROM HUMAN BLOODSTAINS

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The field of forensic science is continually evolving and adapting the latest techniques to be able to analyze the samples obtained from a crime scene. In contrast to whole blood use for paternity testing, biological stains at crime scenes are often exposed to UV (sun) light, humidity, and decay resulting to variable quality and quantity of extractable DNA. In the continuing effort of the UP-PACC DNA Analysis Laboratory to develop DNA Analysis as a tool forensic investigation in the Philippines, the laboratory progressively validates various protocols and procedures for their application in the Philippine setting.

The objective of this study is to determine the length of time and temperatures at which human blood stains can be exposed to yield DNA amplifiable with STR primers. Several 50-microliter blood stains were exposed to varying conditions of temperature (25°C, 27°C, 32°C), and time (3, 7, 14, and 28 days). The DNA from these samples was extracted using a previously valid method of phenol extraction and alcohol precipitation (Kirby, 1992). Sufficient amounts of DNA (70-400 μg/

mL) were recovered after 28 days of exposure. All DNA extracts were amplified at the STR locus HUMFOLP23 (HUMDHFRP2). Apparently, DNA in the blood exposed to temperatures of 25°C-32°C up to 28 days was not significantly degraded at the HUMFOLP23 locus. DNA extracted from bloodstains after 28 days of exposure to different conditions was amplified.

Keywords: human blood, dried bloodstains, validation procedures, DNA analysis. DNA extraction, environmental condition, PCR, HUMFOLP23, DHFRP2, STR

GENERATION AND CHARACTERIZATION OF A RECOMBINANT ANTI-TUMOR HUMANIZED TETRAVALENT ANTIBODY

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Monoclonal antibody (Mab) CC49, a murine IgG1, reacts with the antigen (TAG)-72 expressed in a variety of carcinomas including cancer of the breast, lung, colon, ovary, and others. To reduce the immunogenicity of CC49 Mab in human patients, a humanized CC49 (HuCC49) was generated by CDR grafting. Its relative affinity was 2- to 3-fold lower compared to the murine Mab. To improve the tumor targeting of the HuCC49, we constructed a single gene encoding a single chain consisting of a humanized CC49 diabody attached to human γ1 Fc via the hinge region. The diabody, a bivalent antigen binding structure, was made up of VH/VL and VL/VH domains. In each of the variable domain pairs, the heavy and light variable domains were linked through a short linker peptide, while the two pairs were linked via a 30 residue gly-ser linker peptide to make two antigen binding sites by lateral and noncovalent association of VL of one pair with the VH of the other. Transfectomas expressing the single gene secreted a homodimar of about 160 kDa which reacted to TAG-72, showed cytotoxicity activity, and had a higher functional affinity than HuCC49. This humanized tetravalent antibody molecule is a promising reagent for diagnosis and the therapy of a wide range of human carcinomas.

Keywords: antigen, carcinomas, CDR grafting, diabody, humanized antibody, immunogenicity, monoclonal antibody, recombinant, tetravelent antibody, transfectomas

MULTIPLE ROLES FOR THE N-MYC GNA IN MAMMALIAN CNS DEVELOPMENT

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The N-myc gene belongs to the myc family of cellular oncogenes. It is amplified in many types of human tumors of neural origin such as neuroblastoma and retinoblastoma. The gene product of N-myc is a transcription factor which upon heterodimerization with the Max protein binds to target genes in a sequence-specific manner. To identify the *in vivo* role of N-myc in mice the N-myc gene was specifically ablated in ES cells to produce N-myc targeted mice. Phenotypic analysis of c57Bl/6 embryos at various stages revealed that N-myc homozygous null mutant mice showed defects such as kinky spinal cords with holes or lateral outpocketings in the turnk region and formation of an extracephalic flexure in the dorsal dlencephalon. Neural precursor cell culture studies, immunosaining, as well as RNA *in situ* hybridization analyses using a variety of neural markers all point to multiple roles for the N-myc gene in the formation of the neural tube, organization of the metameric pattern of spinal neurons, development of the cranial ganglia, and in the migration of cephalic neural crast cells.

Keywords: N-myc, knockout mouse, CNS development, cranial ganglia, neural pathfinding, metameric pattern C57Bl/6

PHYSIOLOGICAL RESPONSES OF FOUR MICROALGAL ISOLATES TO CADMIUM

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Four isolates vix., Bat-09 *(Chroococcus)*, Cav-25 *(Desmococcus)*, and CdO-15 *(Chroococcus)* were used to evaluate physiological responses to cadmium treatments of varying levels (i.e., 0.05, 0.5, 5.0, ppm $CdCl_2$) The growth responses of these microalgal isolates were determined through tubidometric analysis and chlorophyll a levels. The uptake of heavy metals by the isolates was determined by Atomic Absorption Spectrophotometry (AAS). All isolates effectively absorbed the heavy metals and uptake increased with concentration within three days. High Performance Liquid Chromatography (HPLC) analyses of protein fractions detected the presence of heavy metal binding polypeptides. This was variable and was not dependent on the concentration of the heavy metals. These isolates are currently being evaluated for bioremediation studies.

Keywords: cadium Chl a, microalgae, AAS, HPLC, polypeptides, bioremediation

HISPATHOLOGICAL ALTERATIONS IN THE INTESTINE OF *Chanos chanos* IN RESPONSE TO MOLLUSCIDE METALDEHYDE

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Metaldehyde is one of the molluscides recommended by the Department of Agriculture-Pesticide and Fertilizer for commercial use to control mollusk, infestation. The effect of metaldehyde on non-target organisms, such as milkfish (Chanos chanos), was studied.

Fingerlings with a mean total length of 5.1 cm were exposed to 3, 30, 60, 150, and 300 mg l^{-1} metaldehyde Porsnail® concentrations for 96 hours. The upper 1/3 of the intestine was fixed and processed for paraffin sectioning. Under light microscopy, sublethal concentrations of metaldehyde induced widening of the lamina propia. Destruction of the intestinal epithelium and detachment of the muscosla layer be-

came visible starting with 30 mg/L, with increasing severity as the toxicant concetration was increased. Hypertrophy of epithelial cells was noted in fish treated with 300 mg l^{-1} metaldehyde.

Keywords: molluscicide, metaldehyde, histopathology, intestine, *Chanos chanos*

GENETIC ANALYSIS OF CHEMICALLY-INDUCED LOSS OF ALBINISM IN MICE

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Albinism is a condition caused by a tyrosinase deficiency and is marked by an inability to form melanin, a dark brown to black pigment responsible for the coloration of the eyes, skin, and hair. In mice, coat color is encoded by genes located in four loci namely; *a* (agouti), *b* (brown), *c* (albino), and *d* (dilute). The wild type C allele encodes the tyrosinase enzyme responsible for melanin production and is dominant over all other mutations at the *c* locus. Albinism thus results from a homozygous (c/c) genotype. We serendipitously generated non-albino mice from both albino parents previously exposed to sodium nitrate when we were originally searching for chemical mutagens affecting the nervous system. All 14 pups of one litter were hyperactive and progressively showed chinchila mottled coat color and black eyes in sharp contrast with their albino (white) father and mother. Subsequent matings of F1 females to albino males revealed that the mutation is a germ-line mutation. The non-albino mice are now being bred to produce inbred and outbred lines for analyzing the mechanisms of nitrate-induce activation of melanogenesis and to identify at which critical point in the melanogenesis pathway did the chemical mutagen interfere in the normal course of melanin suppression in the original albino stock.

Keywords: albino, ICR, mouse, melanin, nitrate, tyrosinase, coat color.