Trans. Nat. Acad. Sci. Tech. Philippines 22: 233-259 (2000).
ISSN 0115-8848

BIOLOGICAL SCIENCES

24. ECOLOGICAL RISK ASSESSMENT FOR HIGH ENVIRONMENTAL QUALITY

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The need to quantify the risk of adverse environmental consequences to human and non-human resources as caused by ecological hazards has generated the development of risk assessment methodology. It is a necessary instrument for a quantitative environmental impact development project. Of the many that are presently existing, most if not all, are so mathematically complicated that they cannot be easily followed and adapted by administrators, scientists, and resource management practitioners. This study represents an attempt to formulate a simple and easily usable treatment of the methodology.

It is the main goal of this study to promote high environmental quality and, to attain this, the following specific objectives are adopted:

1. to evaluate an ecological risk assessment model that measures the probability of occurrences of adverse environmental impacts as caused by ecological stresses and
2. to use this model in assessing the acceptable concentration as the basis of developing mitigating options.

Environmental risk is determined as:

$$ER = (Obs/Exp)/S \qquad \text{Eq. 1}$$

Where *ER* = coefficient of relative environmental risk factor
Obs = number of affected cases observed in the exposed group
Exp = number of affected cases expected in the exposed group
S = ecological stress measured by C • . • T:

C = concentration in units of mg / cu m
I = standard intake in cu m
T = number of days of exposure

The probability of occurrence of adverse environmental impacts is further evaluated as:

$$P = E\,R \times S \times (\Sigma\, ER/n) \qquad \text{Eq. 2}$$

Where P = probability of occurrence of adverse impacts
R = same as defined in Eq. 1
S = same as defined in Eq. 1
n = number of ER rates

The model was tested for prediction of adverse consequences in several studies, such as pesticide use, soil erosion control, mortality rates due to cancer, and morbidity rates due to water pollution. Pesticide use and soil erosion studies used a quasi-experimental design, comparing the exposed and non-exposed groups on the effects of the intervention. The last two studies used secondary data.

The assessments show results that indicate the model as a good predictor of adverse responses which are as good as the standard rates for the general population. The evaluations of acceptable concentrations further are consistent with what are prescribed by public agencies.

The ERA model provides a significant input to decision-making related to developing the most appropriate regulatory actions for human exposures, industrial plant emissions, ambient air and water exposure, and mitigation options formulated to control serious adverse environmental effects.

Key words: ecological risk assessment (ERA), high environmental quality, environmental risk factor, ecological stress, probability, concentration, exposure, time of exposure

25. MULTIPLE SHOOTING IN COTYLEDONARY NODES AND *Agrobacterium*-MEDIATED TRANSFORMATION IN *Pterocarpus indicus* WILLD. (FABACEAE)

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Pterocarpus indicus Willd. (Fabaceae) locally known as narra, is an indigenous tree in Southeast Asia. Its wood gives one of the best materials for furniture making. It is also a good source of red dye, has some medicinal uses, and is one of the multi-purpose trees. However, propagation of the plant by seeds is beset by problems involving their being prone to insect attack and various diseases. Thus, a manageable and efficient method of in vitro propagation is necessary to supplement the conventional method of propagation and to improve its germplasm successfully to genetic engineering.

Germination and survival rates and the response to the formation of axillary shoots in cotyledonary nodes were compared between two provenances of prickly narra. Laguna and Manila provenance seeds germinated at 39.16% after four to seven days and 50.00 after two to five days, respectively. With a survival rate higher in Manila provenance, the total node explants inoculated were 52 while in the Laguna provenance, only 26 were available for use. Murashige and Skoog (MS) and Woody Plant Medium (WPM) supplemented with 0.90 mg/L BA alone or in combination with 0.186 mg/L NAA did not have a remarkable effect on the growth of the excised embryos. However, the formation of axillary shoots on cotyledonary shoots and the quality of seedling growth were both affected by the different concentration and combination of cytokinins added to the MS multiplication medium. Seedling growth in Manila provenance was robust; Laguna seedlings were frail. Cotyledonary nodes from Laguna produced the usual 2 axillary shoots per node while in the Manila provenance, three to seven axillary shoots per node were observed in the following treatments: MS + 2.00 mg/L BA + 2.5 mg/L thidiazuron (TDZ), MS + 2.00 mg BA + 2.5 mg/L zeatin + 2.5 mg TDZ, MS + 2.00 mg/L BA + 5.0 mg/L zeatin, MS + 10.00 mg zeatin, and MS + 10.00 mg/L LTDZ. This is the first report on multiple axillary shooting in cotyledonary nodes of narra and its transformation using *Agrobacterium*.

Some tissues were successfully transformed with *A. tumefaciens* LBA 4404 which harbored a binary vector, p81121 (Clontech, Palo Alto, CA) with genes for B-glucuronidase (GUS) and neomycin phosphotransferase (NPTII). The cotyledons were the best tissues that responded to transformation as shown by the appearance of a blue color in the transactions under the treatment of X-GLUC (5-

bromo-4-chloro-3-indolyl-β-D-glucuronide) solution. Further tests with the use of PCR-based technology are needed to supplement the result of the histochemical test for GUS activity.

Key words: cotyledonary nodes, Agrobacterium, transformation, *Pterocarpus indicus* Willd., narra, provenance, multiple shooting, axillary shooting, zeatin, thidiazuron.

26. EMBRYONIC DEVELOPMENT OF "HANGA"

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"Hanga" or *Pittosporum resiniferum* Hemsl. is a potential alternative source of energy because of the petroleum-like properties of the oil from the fruit which is comparable to petroleum due to the presence of dihydroterpene and n-haptane (Bacon 1909; Noble 1978). The study was conducted to trace and describe the different stages of development of the embryo from globular to the mature stage. The modified clearing technique using NaOH and chloral hydrate was used. Seeds were removed from the fruits and the embryos in the seeds were isolated. The embryos were measured with a micrometer eyepiece using a BH-2 Olympus epifluorescent microscope. Morphological and anatomical descriptions were used as criteria for classifying the embryos in different stages. Stage 1 had embryos which were small and globular in shape. In stage 2, the embryos were in the early-heart shape, with cotyledons developing. In stage 3, the embryos were in the mid-heart shape with developed cotyledons. A suspensor, at the base of the embryo was observed. In stage 4, the late-heart-shape, the cleavage between the cotyledons was deeper and the cotyledons more rounded at their tips. The primary tissues (protoderm, procambium, and the ground meristem) were well defined.

One embryo per seed was noted. Approximately 80 to 90% of the seeds dissected showed the presence of an embryo. The presence of an embryo and its developmental stage are not directly related to seed size.

Key words: embryo, suspensor, procambium, dihydroterpene, n-heptane, cotyledons, protoderm, ground meristem Hanga, *Pittosporum resiniferum*

27. THE CULTURE OF *Kappaphycus alvarezii* (DOTY) AT THE THREE DIFFERENT WATER LEVELS IN THE MARINE WATERS OF NORTHERN POBLACION, SAN FRANCISCO, CEBU

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Camotes Island is noted for its Camotes Sea, one of the fishing grounds in the Philippines. It is composed of three islands namely: Pacijan Island where the municipality of San Francisco is located; Poro Island, which is divided into two municipalities, Poro and Tudela; and the smallest is Ponson Island where the municipality of Pilar is found. The fishermen in the area are complaining on the decline of their catch. The livelihood of the majority of the inhabitants are fishing and farming and they are becoming poorer because at present they have no alternative livelihood to augment their income.

This study was undertaken to find out what level of water the *Kappachycus alvarezzi* locally known as "gozo" will grow in the marine water of San Francisco, Cebu using the randomized completely block design (RCBD), with the increase of weight as indicator for growth. There were three treatments in the study with three replicates such Treatment 1 represents the surface layer; Treatment 2 represents the middle layer, and Treatment 3 represents the bottom layer. Bamboo rafts were used to hold the plants at the three different levels. Seedling were selected from the midpart of the whole plant up to the tip. Plant samples were weighed every 15 days for 45 days.

The area parameters are salinity, 32-39 parts per thousand (ppt); water temperature, 31-32°C; water velocity, 4 to 8 seconds per meter, water depth, 3 meters to 3.75 meters, and water transparency, 3 to 3.5 meters.

Results based on the mean weight of the plant after 45 days show that surface layer has 1,395 grams; middle layer 1,052.50 grams, and the bottom layer is 834.44 grams.

Analysis of Variance (ANOVA) tables shows that there is no significant difference in all the treatments meaning all the levels are suitable for gozo culture.

Key words: *Kappaphycus alvarezii*, Camotes Sea

28. VARIATION IN GINGER *(Zingiber officinale* Rosc.) AND RELATED TAXA USING ISOZYME PATTERNS

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Ginger (*Zingiber officinale* Rosc.), an aromatic herb with an underground rhizome, belongs to the family Zingiberaceae. Related taxa such as *Zingiber zerumbet* (L.) Smith, *Z. purpureum* Rosc. (syn. *Z. cassumunar* Roxb.), *Curcuma* spp., and *Alpinia* spp. have likewise been reported to have some economic and medicinal importance. Earlier works done on *Zingiber* and other members of the ginger family, which largely deal with chemical, taxonomic, and pharmacognostic studies, revealed genetic variation for several morphological characters. However, zingiberaceous plants possess a limited number of morphological features useful in classical taxonomic evaluations, making species level identification of these plants difficult in the absence of the parts, especially floral structures, that bear these key traits. More recent biochemical studies on two genera, *Zingiber* and *Curcuma*, using differences in isozyme patterns have shown interspecific variations for a number of enzyme systems (Ibrahim, 1996); however, such diversity has not been critically evaluated. This project was thus undertaken to determine if there is significant interspecific and intraspecific variation shown through isozyme banding patterns and to ascertain if isozyme analyses could prove useful as taxonomic markers in this group of plants. To do this, protein extracts from mature leaves of 10 accessions of ginger and related taxa (7 *Zingiber*, 2 *Languas*, and 1 *Curcuma*) from at least 3 regions of the Philippines were subjected to electrophoretic techniques using polyacrylamide gels to generate isozyme banding patterns for 6 enzyme systems: esterase (EST), peroxidase (PRX), acid phosphatase (ACP), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), and catalase (CAT). Clustering methods using the Multi-Variate Statistical Package software were performed to analyze the variation in banding patterns. It was found that EST, PRX, and ACP showed significant interspecific as well as intraspecific variation among the accessions. GOT and MDH revealed interspecific variation but did not substantially delineate between samples of the same species. CAT, on the other hand, exhibited little variation in all the accessions. Clustering of the *Zingiber officinale* samples was observed to be geographically affected, with samples from the north (Luzon) clustering together and those from the south (Visayas and Singapore) forming another group. These results suggest that the use of isozyme banding patterns as taxonomic markers may be possible depending the enzyme systems being used.

Key words: Zingiberaceae, isozyme, interspecific variation, intraspecific variation, electrophoretic techniques, clustering methods

29. CORRELATION OF PANDANUS ALKALOIDS TO THE TAXONOMY OF PANDANACEAE

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Leaves of *P. amaryllifolius*, *P. dubious*, *P. simplex*, *P. laevis*, *P. veitchii*, and *P. variegatus* and the species described as a cross between *P. nobilis* and *P. vidalii* collected from Luzon were detected to contain alkaloids by the Culvenor Fitzgerald field test. *P. tectorius* Sol. On the other hand did not show a reproducible response to the alkaloid field-test. *P. laevis*, *P. veitchii*, and *P. variegates* were all identified to be cultivated varieties of *P. tectorius*.

The first novel alkaloid, pandamarine, from *P. amaryllifolius* of the subgenus *Kurzia* was reported in 1992 by the team from UST RCNS. These were further substantiated by the report of 3 more new alkaloids from the same plant. Works by Garson and Sjaifullah on the same plant obtained from Indonesia reveal more new alkaloids. More recently, this researcher and the group of Prof. N. Aimi of Chiba University isolated more new alkaloids from plant materials obtained from the Philippines and Thailand. *P. dubius* Sprengel, a species classified under the subgenus, *Rykia*, gave similar alkaloids like that from *P. amaryllifolius*. The results establish the basic structure of the *Pandanus* alkaloid to be C_9NC_9. *P. nobilis* x *P. vidalli* yields vomifoliol (blumenol A) suggesting the occurrence of a false positive reaction with the alkaloid-precipitating reagent. No alkaloid was isolated form *P. tectorius* that responded with the field test. The cultivar *P. variegates* also did not produce any alkaloid despite its initial response to the field test. The detected alkaloids in *P. laevis* and *P. veitchii* are now being studied. True alkaloids were found present in species belonging to the subgenera *Rykia* and *Kurzia*. The subgenus *Pandanus* where *P. nobilis* x *P. vidalii*, *P. variugatus*, and *P. tectorius* belong to was found to give false positive tests for alkaloids. All of these observations proved to be important for the taxonomic classification of these plants. This paper will present the relationship of the alkaloids found to the Pandanaceae taxonomy.

Key words: Pandanaceae, chemotaxonomy, alkaloids, *Pandanus*

30. LITTORAL FISHES FROM A SEAGRASS AREA IN SAMAL ISLAND, DAVAO DEL NORTE, PHILIPPINES

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Monthly fish samplings were conducted in a seagrasss coastal area in Samal Island, Davao del Norte, Philippines, from March 1998 to April 1999 to determine the composition and abundance of fish populations associated with seagrass in the area. A customized beach seine was used to collect fish during the day and at night. A total of 159 fish species comprising 43 families were caught. The pipefish, *Syngnathoides biaculeatus*, and the pufferfish, *Canthigaster bennetti*, were dominant both in total counts and biomass during the day, while at night, the dominant fishes were the rabbitfish *Siganus spinus*, *Apogon coccineus*, *A. fraenatus*, *Cheilodipterus macrodon*, and *C. quinquelineatus*. Fish total abundance and biomass were significantly higher during the day than at night. The dominant species based on total counts were mostly carnivores (39.6%), followed by planktivores (33.8%), herbivores (24.2%), and omnivores (2.6%). Fish density in the study area was 0.46 ± 0.0086 ind m-2 and 3.89 ± 0.7183 g wet wt m^{-2}.

Key words: seagrass fishes, Samal Island, Davao del Norte, pipefish, pufferfish, rabbitfish, carnivores, planktivores, herbivores, omnivores

31. THE EFFECTS OF SALINITY ON SURVIVAL AND GROWTH OF TILAPIA *Oreochromis niloticus* EXPOSED AT VARIOUS AGES

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Tolerance of tilapia, essentially a freshwater fish, to artificial seawater acclimation based on survival and growth at 1, 11, 21, 31, and 41 days post-hatching was determined. Optimum survival (100%) was observed in fry exposed to seawater very soon after hatching. Survival rates of 56.67, 76.67, 90, and 93.33% were observed for fry acclimated at 11, 21, 31, and 41 days post-hatching, respectively; indicating a trend towards increased tolerance with age. There was no further statistically significant deviation ($P<0.05$) as compared to survival of non-acclimated control (96.67%) starting 31 days post-hatching. There were no significant effects on the growth of fish surviving through 61 days post-hatching.

Key words: tilapia, artificial seawater, acclimation, fry, hatching

32. REPRODUCTIVE DEVELOPMENT OF THE SUPERMALE (YY) TILAPIA *(Oreochromis niloticus)*

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Histogenesis of the reproductive system of supermale (YY) tilapia and XY tilapia reared at the Central Luzon State University was analyzed using the paraffin technique. In the course of development, the primordial germ cells appeared at the same age in YY and XY at 8 days posthatch. These cells which were larger in the YY (1.85 μm) that in the XY male (0.9 μm) later established themselves in the gonadal anlage by days 9 to 22. The lobules appeared earlier in the YY at day 15. Blastema of the reproductive duct appeared in the YY at day 23 and in XY at day 27. By day 79, meiotically active cells were abundant in both groups. By day 95, the YY fish showed mature sperm cells in the fully differentiated testis as compared to day 105 in the XY fish. The supermale consistently demonstrated bigger testis, thicker somatic tissues, more spermatogenic cells, and more advanced developmental stage than XY fish of the same age. Germ cell and nuclear size in the YY and XY fish were not significantly different by statistical analysis although the general trend was bigger spermatogenic cells in the supermale tilapia. Anova (a 0.05) showed significant difference in the size of the testis, spermatocysts, and vas deferens. The study showed that with the same rearing conditions and same age, the larger supermale tilapia has superior reproductive capacity with its larger testis and ducts and faster histogenesis, differentiation, and spermatogenesis.

Key words: supermale (YY) tilapia, *Oreochromis niloticus*, histogenesis, reproductive development

33. MORPHOANATOMY OF *Vivipara costasa* Quoy and Gaimard (Mollusca: Viviparidae) DURING EARLY DEVELOPMENT

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The early development of the freshwater gastropod snail, *Vivipara costata* Quoy and Gaimard, was characterized by observing the live embryos in the following series: 1-cell stage, 2-cell stage, 4-cell stage, 8-cell stage, morula stage, blastula stage, gastrula stage, trochophore stage, early veliger stage, veliger at start of torsion, veliger at 90° torsion, veliger at 120° torsion, veliger at post-torsion, and juvenile stage. The trochophore larval stage was subdivided into 3 substages with different sizes and distinctive characteristics.

The fertilized egg contained a small amount of yolk making the embryo transparent until late veliger stage. Cleavage was spiral. Cleavage cavity and polar lobes were absent. The blastula had a wide blastocoele. Gastrulation was by invagination. There was reduced ciliation in the prototroch of trochophore larva and apical tuft was absent. The veliger larva was of the dominant larval type.

The actual age of the embryos was not determined in this study because they were contained within the brood pouch of the mother and so the different stages were categorized based on their morphological features and relative sizes. The derivatives of the three germ layers namely, the ectoderm, mesoderm, and endoderm, were determined by histological sections using the paraffin method.

The anatomical shifting in the positions of some larval organs relative to their point of origin was attributed to torsion during differential growth. These changes included the shifting of: the heart, kidney, and ureter contained in the visceral sac from the right to the left side; the anus from left to right; the mantle cavity from posterior to anterior; and the crossing over of the right and left intestinal ganglia.

Key words: *Vivipara costata*, morphoanatomy, embryogenesis, organogenesis, trochophore, veliger, torsion, viviparous, histology, conchology

34. DIVERSITY OF THE MOLLUSCAN GASTROPODS, *Terebralia sulcata* AND *Cerithidea cingulata* IN TWO MANGROVE AREAS IN CATANDUANES, BICOL REGION

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Typhoons serve as major stresses in the mangrove communities of the island province of Catanduanes, Bicol region. Such disturbances can shift and change the structure of floral and macrofaunal species found in the mudflats of mangrove areas.

From October 21-22, 1998, a super-typhoon damaged the mangrove areas of the southern portion of Catanduanes, particularly in the municipalities of Bato, Virac, and San Andres. In order to understand possible changes in the diversity of bottom dwelling macrofauna, quantitative surveys were conducted from 1998-1999. This was limited to the molluscan gastropods, *Terebralia sulcata* and *Cerithedia cingulata* (Family Potamididae) The quantitative sampling of the two species of molluscan gastropods was done along a series of quadrats established in two study sites. Samples were collected by dividing a square sheet wooden frame of 1-mx 1-m into 4 parts. *T. sulcata* has very unusual abundance towards the seaward fringes, while *C. cingulata* is abundant towards the landward fringes. The former tends to have preference for high salinity seawater compared to the latter which mostly aggregate under Nipa palms and mangrove trees where the salinity is less.

The Panganiban mangrove area in the north is characterized by river-dominated allochthonous setting and low tidal range as evidenced by the narrow tidal changes during the day. Oco river discharge of freshwater and sediments leads to the deposition of terrigenous silts. On the other hand, the mangrove area in Virac shows a composite river and wave-dominated setting. Sto. Domingo-Pajo river provides the freshwater supply and sediments. The mangrove setting in this capital town represents a combination of high wave energy and river discharge. The sand that is debounced by this river is distributed by waves forming sand sheets.

Surveys conducted from July to September, 1998, showed the mean density values of 115.42 in Panganiban and 123.72 in Virac for *T. sulcata*. In January-February, 1999 (2 to 3 months after the super typhoon ravaged the province) field surveys were again conducted in the said locations using permanent transects and quadrats showed significaotly lower mean density values of 75.42 in Panganiban and 58.25 in Virac from 100 quadrats. In December, 1999, another field survey was conducted in Virac and very low densities of both *T. sulcata* and *C. cingulata* were obtained. The decreasing density of the molluscan gastropods in consideration could be attributed to constant flooding in the mangrove areas which disturbs the macrofaunal communities in the province. Habitat change involving changing

patterns of coastal landforms and geomorphic processes could explain these changes in the diversity of molluscan gastropods.

Key words: mollusks, gastropods, diversity, density, mangroves, Catanduanes

35. ELECTRON MICROSCOPE ANALYSIS OF SEXUAL INDUCTION IN A FISSIPAROUS PLANARIAN, *Dugesia ryukyuensis*

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Minced flatworm, *Bdellocephala brunnea*, was fed to the asexual mixoploid biotype *Dugesia ryukyuensis* (Okinawa-Hiroshima strain) to determine if the asexual worm could be sexualized. Six weeks feeding led to full development of the reproductive organs of *Dugesia*. Ultrastructural observations showed features previously unreported in worms of the same group.

Key words: *Dugesia ryukyuensis*, Bdellocephala brunnea, sexualization, asexual worm, mixoploid biotype

36. GILL PARASITES OF *Terapon jarbua* FORSKAL, FROM LINGAYEN GULF

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Terapon jarbua (Forskal), locally known as "bagoong", inhabits inshore waters and brackishwaters and enter freshwater bodies. It is found practically all over the Philippines where it is utilized for food. Examination of the gills of samples obtained monthly from Lingayen Gulf in La Union Province, from January to December 1998 resulted in the recovery of parasites. A total of 162 specimens

ranging from 6.0 to 21.2 cm were examined. The most prevalent parasite was a monogenetic fluke, the others found were crustaceans. The parasite species recovered, prevalence (%), and intensity of infection (number per infected host) were: *Diplectanum secundum* (Tripathi) (87%, 1-135), *Caligus* sp (59%, 121) Caligus pelamydis (Kroyer) (38%, 1-9), *Neobrachiella chevreuxii* (van Beneden) (8%, 1-7), *Chalimus* larvae (24%, 1-7), and larvae of *Gnathia* sp. (7%, 1-7). For both the monogenean and crustacean (as a group) parasites, no significant seasonal differences in prevalences of infection were observed. However, prevalences of infection were significantly higher with bigger specimens than with smaller one.

Key words: *Terapon jarbua*, Lingayen Gulf, gill parasites, *Diplectanum*, *Caligus*, *Neobrachiella*, *Chalimus*, *Gnathia*, monogenea, crustacea

37. ISOLATION OF INDIGENOUS BACTERIA FOR THE DEVELOPMENT OF PROBIOTICS IN THE BIOCONTROL OF CLINICALLY IMPORTANT PATHOGENS

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Increasing resistance of pathogenic microorganisms to antibiotics is a major public health problem globally. Resistance of pathogenic microorganisms is leading to increased death and illnesses with consequential expenditure. The use of antibiotics for bacterial infections has become ineffective because of the development of bacterial resistance. This has led to the isolation of probiotics that are most likely to inhibit and control the growth of clinically important pathogens.

A total of twenty bacterial isolates were randomly collected from marine environments in Panay Island, Philippines. Inhibitory activities of these bacterial isolates against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus* spp. Were examined using cross streaking procedure and modified sensitivity test, in vitro. Eleven isolates showed a moderate inhibitory activity against *S. aureus*, three against *P. aeruginosa*, and two against *Streptococcus* spp. Two of the twenty isolates, coded MU4 and SU5, showed a strong inhibitory activity against *S. oureus* using the modified sensitivity test while no significant effect was observed against *P. aeruginosa* and *Streptococcus* spp. Characterization of the bacterial strains using morphological classification revealed that strains MU4 and SU5 were gram-positive bacilli and gram-negative bacilli, respectively.

Key words: pathogenic microorganisms, probiotics, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp., cross streaking, antibiotics, gram-positive bacilli, gram-negative bacilli

38. Δ^4-3-KETOSTEROIDS FROM *Morinda citrifolia* L. AS POTENTIAL INHIBITORS OF *Mycobacterium tuberculosis* H37Rv

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Tuberculosis, a disease once thought to be under control due to the discovery of effective chemotherapeutic agents, has come back with a vengeance claiming the lives of nearly 3 million people in 1995 worldwide. According to WHO, the Philippines is ranked as the fourth country with the highest incidence of TB in 1995. This ancient scourge, which is now a global epidemic, prompted the search for compounds with antitubercular properties. One promising plant source is *Morindia citrifolia* L. (Rubiaceae). Ethnomedical studies in Hawaii, where this plant is called "noni", reported that the locals used a concoction from this plant to treat tuberculosis.

Preliminary in vitro screening conducted on the alcoholic leaf extract of this plant indicated significant inhibitory activity against the virulent *Mycobacterium tuberculosis* $H_{37}Rv$, the causative agent of TB. Bioassay-guided purification of the hexane-soluble constituents of the alcoholic extract of *M. citrifolia* L. leaves yielded a fraction that exhibited a minimum inhibitory concentration of <2.0 μg/mL against the test organism. Reversed phase HPLC purification of this fraction by isocratic elution using MeOH as mobile phase yielded two Δ^4-3-ketosteroids. Elucidation of structures was carried out by infrared and nuclear magnetic resonance spectroscopy and mass spectrometry and comparison with known data. These were identified as stigmasta-4,22-dien-3-one and stigmast-4-en-3-one.

Key words: tuberculosis, chemotherapeutic agent, *Morinda citrifolia* L., Rubiaceae, noni, in vitro, *Mycobacterium tuberculosis* $H_{37}Rv$, Δ^4-3-ketosteroids, stigmasta-4,22-dien-3-one, stigmast-4-en-3-one

39. PIGMENTED OFFSPRING OF ALBINO MICE: SCREENING FOR GAIN-OF-FUNCTION MUTATIONS IN FIVE EXONS OF THEIR TYROSINASE GENE BY MULTIPLEX PCR-SSCP ANALYSIS

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Albinism is a condition marked by the absence or inability to produce melanin in the body. We previously reported the serendipitous generation of pigmented (agouti) mice from albino parents. Since albinism results from loss-of-function mutations in the gene tyrosinase which encodes the key enzyme that converts phenolic compounds in the body into melanin, was screened for gain-of-function mutations in the tyrosinase gene of these agoutimice. As a rapid and sensitive procedure to detect point mutations in the gene, was simultaneously amplified multiple exons by multiplex PCR and performed single-strand conformational polymorphism (SSCP) analysis. We discuss the conditions for multiplex PCR-SSCP and the applications of this technique to screen for subtle point mutations in genes which cannot be determined from the length of PCR products alone when primers flanking exons are used.

Key words: tyrosinase, melanin, albino, multiplex PCR-SSCP, loss-of-function mutation, gain-of-function mutation

40. *Schefflera odorata* INHIBITOR OF MARK ACTIVATION IN CULTURED AIRWAY SMOOTH MUSCLE CELLS

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Mitogen activated protein kinase (MAPK) is an enzyme belonging to the group of phosphotyrosine kinases. Phosphotyrosine kinases are enzymes that are involved in cell growth and proliferation. These enzyme are activated by phosphorylation. The effect of the leaf extract of *Schefflera odorata* on MAPK activation in airway smooth muscle (ASM) cells was investigated. Thymidine incorporation assay was done to determine if *S. odorata* can inhibit DNA synthesis of ASM cells. Fetal calf serum (FCS) was used to stimulate DNA synthesis in ASM cells. FCS-stimulated DNA synthesis was inhibited by 50% when treated with 100 µg/mL. *S. odorata* extract. Almost 100% inhibition of DNA synthesis was observed when the cells were treated with 200 µg/mL. The effect of *Schefflera odorata* on phosphotyrosine kinases in ASM cells was determined by SDS-PAGE and Western Blotting. FCS was used to stimulate the activation of these enzymes. Immunoblot of the ASM cell lysates treated with FCS and *S. odorata* extract showed concentration dependent inhibition of band formation at 205,112, and 42 kDal. The effect of *S. odorata* on MAPK activation was determined by performing two assays, MAPK SDS-PAGE mobility shift and MAPK peptide assay. In SDS-PAGE mobility shift assay, the activation of MAPK is indicated by its retarded mobility upon SDS-PAGE, as observed in ASM cells treated with FCS. However, this mobility shift was not observed when the cells were treated with FCS and 100 µg/L of *S. odorata*. No band was formed at all when the concentration of *S. odorata* was increased to 200 µg/mL. The result in the MAPK mobility shift assay was confirmed by the other assay, the MAP peptide assay.

Key words: MAPK, ASM cells, cell growth, cell proliferation, phosphotyrosine kinases, thymidine incorporation assay, MAPK SDS-PAGE mobility shift assay, PAK peptide assay, western blotting, immonoblot

41. THE EFFECT OF *Tinospora rumphii* BOERL ON OVARIAN 3β-HYDROXYSTEROID DEHYDROGENNASE ACTIVITY IN RATS

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The antifertility affects of *Tinospora rumphii* Boerl. ("makabuhai") has been demonstrated. In this study, the effect of *Tinospora rumphii* methanol extract from air-dried stems on ovarian 3β-hydroxysteroid dehydrogenase activity in both virgin and mated (pregnant) rats was investigated. The enzyme is important in the biosynthesis of reproductive hormones. The drug in single doses of 2μ/kg, 4μ/kg, and 8μ/kg was injected into the ovarian bursae at diestrus in virgin rats and at 5th, 9th, and 18th day of pregnancy in mated rats. Spectrophotometric determination of enzyme activity assayed levels of NADH at 4, 8, 12, 24, and 48 h after treatment. Assay results showed significant reduction of enzyme activity within the first 24 hours in all the *Tinospora* treated rats except those treated with the lowest dose. The effect of 8μ/kg was comparable to that of 2μ/kg 17αhydroxy-6α-methoxyprogesterone acetate (positive control). There was recovery of enzyme activity after 48 hours. Nevertheless, histopathological examination on the ovaries revealed that many follicles in the Tinospa-treated rats had become atretic just like in the positive control but *Tinosporaca* used 72% reduction in fertility and some generated fetuses (and not 100% reduction and no degenerated fetuses as in the positive control).

Key words: herbal drug, atretic ovarian follicles, anti-fertility drug, steroid biosynthesis, 3b-hydroxysteroid dehydrogenase

42. DESIGN OF A QUANTITATIVE BEHAVIORAL TEST FOR HYPERACTIVITY IN MUTANT MICE

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We previously reported the serendipitous generation of pigmented mice from albino parents orally treated with a suspected mutagen, sodium nitrate. The gain-of-function mutation resulted in the birth of agouti as well as black mice in the

otherwise albino population and this mutation was stably transmitted to the germline. The pigmented F_1 agouti mice were further observed to exhibit hyperactive behavior relative to same-age albino mice raising possibility that the hyperactive behavior may be linked to the coat color phenotype. To test this possibility, we developed a quantitative hyperactivity test in conjunction with molecular and genetic studies being done. Here, we describe the experiment set up we developed to test for hyperactive behavior in mice. We show that our device could be used not only to quantify the endurance of the mouse given a particular test situation, but also to determine whether the mouse respects spatial boundaries.

Key words: hyperactive mice, gain-of-function mutation, hyperactivity test, sodium nitrate, pigmented mice

43. HEPATOTOXICITY OF METALDEHYDE

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The hepatotoxicity of the molluscicide metaldehyde on non-target organism such as milkfish was determined through acute exposure. Chanos chanos fingerlings were exposed to varying concentrations of Parsnail^R, namely: 3, 30, 60, 150, and 300 mg L^{-1} and liver samples were processed for paraffin sectioning.

Light microscopy showed hypertrophic depatocytes in all sublethal treatments. Except for liver sections at 3 mg L^{-1}, all other treatments revealed sections with pyknotic nuclei and chromatin clumping > Cytoplasmic vacuolation and loss of cellular architecture were evident in liver sections of fish exposed to 300 mg L^{-1}.

Key words: hepatotoxicity, PorsnailR, molluscide, milkfish, fingerlings.

44. ASSESSMENT OF THE ACUTE TOXICITY OF SURFACTANTS (LAS AND CFAS) USING SELECTED SPECIES OF FISH

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Despite the wide use of surfactants in the Philippines, data on the environmental toxicity of these compounds to tropical organisms is scanty. The majority

of the chronic and sublethal toxicity databases that are available are limited to studies in temperate areas involving only a few commercially important types of surfactants. Hence, the present study aims to determine the acute toxicity of LAS and CFAS on three local freshwater fish species – common carp *(Cyprinus carpio,* Linneus 1758), guppy (*Poecilia reticulata)* and tilapia *(Tilapia nilotica)* – under static-renewal conditions (12h-replacement of test solution).

A series of 96-h range-finding tests was performed to determine the critical concentrations Subsequently, 96-h definitive tests was done in order to determine the 96-h (LCSO (mg/L). A series of reference toxicity tests with copper sulfate were performed in parallel. Test solutions were analyzed every 12 h to determine actual surfactant concentration using direct injection negative ion Electrospray Ionization-Mass Spectrometry (neg ESI-MS).

The results of the chemical analysis of the test water showed that CFAS was partially degraded after 6 h and is completely degraded after 12 h while LAS concentration remained the same even after 12 h. The rates of change of both LAS and CFAS concentration-response curves were similar and very shallow implying that large decreases in concentration will only bring about small decreases in toxicity. This further implies a possible similarity in the mode of toxic action of the two compounds on the test organisms. The results of the definitive toxicity tests showed that CFAS was found to be 2 to 3 times more toxic than LAS to all three species. Tests with the reference toxicant (copper sulfate) showed that the rank order of sensitivity among the test organisms was as follows: most sensitive was carp, followed by guppy, with tilapia as the least sensitive. In the absence of a standardized toxicity test procedure in the Philippines that can be used or testing in the aquatic environment, the procedure developed here can be used for future monitoring studies.

Key words: surfactant toxicity, LAS, CFAS, toxicity tests, carp, guppy, tilapia

45. GENERAL PROTEIN BANDING PATTERNS OF THE FRESHWATER PRAWN, *Macrobrachium lanceifrons,* COLLECTED FROM VARIOUS SITES IN LAGUNA LAKE

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This study was undertaken to gather an overall molecular fingerprint of the fresh water prawn, *Macrobrachium lanceifrons,* using general protein banding patterns. Prawn samples collected from different sites in Laguna Lake were analyzed through sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SDS-PAGE). General protein bands showed variation in terms of mobilities, intensities, and individual patterns. However, a general banding pattern may be deciphered from all samples analyzed; groups of cathodal and anodal protein bands are apparently separated in the prawn. Differential expressions, of proteins are reflected in minor individual variations in banding patterns observed.

Key words: freshwater prawn, Laguna Lake, *Macrobrachium lanceifrons*, SDS-PAGE, protein banding pattern, polyacrylamide gel electrophoresis, molecular fingerprint, differential expression, cathodal proteins, anodal proteins

45. PURIFICATION, CHARACTERIZATION, AND ANTIMICROBIAL SPECTRUM OF A BACTERIOCIN FROM FERMENTED SAUCE ISOLATE

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Bacteriocins are antimicrobial compounds that recently captured the interest of various industries because of their great potential of being effective natural preservatives. Some desirable characteristics are wide range of pH and heat stability, tastelessness, digestibility due to their proteinaceous nature. They have already been consumed for a long time in foods such as fermented dairy and meat products. There are reported types that prevent listeriosis and histamine poisoning and some are useful as genetic markers in the food-grade cloning and expression systems. Nisin is the well characterized and the only legally approved bacteriocin as preservative. It is classified as an additive generally regarded as safe (GRAS). Therefore, knowledge of the properties of known and new bacteriocins will help in hastening the acceptability of bacteriocin-treated products and promote their utilization.

In this study, a bacteriocin from *Pediococcus acidilactici* isolated from fermented sausage was extracted employing a current technique of pH-dependent cell adsorption-desorption of proteins. The desired metabolic product was first adsorbed onto the cell surfaces at pH 5.0 and cells removed from the culture medium by centrifugation. It was selectively released at pH 2.0 and dialyzed to remove acid and salts. Purification under served phase-HPLC resulted in a yield up to 5.76×10^5 times.

Mass spectrometry gave a molecular weight of 4699 Dal, a little higher than previously reported pediocins. The bacteriocin was heat tolerant when exposed to pasteurization setting (61.8°C, 30 min and 71.5°C, 15 sec), boiling temperatures at different time intervals and autoclaving conditions. It was also stable under a wide pH range (pH 3 to 9). Well-diffusion assay showed antimicrobial activity against *Listeria monocytogenes*, a Gram-positive food pathogenic bacterium and some lactic acid bacteria such as *Lactobacillus plantarum*, *Leuconostoc mesenteriodes*, and *Enterococcus faecalis*.

Partial characterization of the basteriocin showed promising features as possible antimicrobial additive in both processed and minimally processed products.

Key words: bacteriocin, *Pediococcus acidilactici*, *pediocin*, purification, antimicrobial spectrum, preservative, adsorption-deposition, well-diffusion assay, anti-*Listeria*, food additive

47. DNA FINGERPRINTING OF COBALT-60 GAMMA RADIATION-INDUCED VARIANTS OF FOLIAGE PLANTS USING AFLP-PCR

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DNA fingerprinting of cobalt-60 gamma radiation-induced variants of a foliage plant (*Murraya exotica*) was studied using amplified fragment length polymorphism (AFLP) coupled to polymerase chain reaction (PCR) technology. Development of the AFLP-PCR protocol was established for variants of *Murraya* and *Dracaena* where polymorphisms are observed using specific selective nucleotide primer pairs to produce unique DNA fingerprints electrophoresed in denaturing acrylamide gel. Genomic DNA used in fingerprinting study was extracted from leaves of a stable mutant of *Murraya exotica* at M_1V_8 vegetative stage. AFLP-PCR amplified fragments produce specific DNA fingerprints for each variant plant that could be used to identify gamma radiation-induced polymorphisms. Genetic markers induced by gamma radiation and observed in AFLP DNA fingerprints were documented against morphological changes of the variant foliage and encoded in the database for *Murraya exotica*. Graphic computer database serves as specialized catalogue of variant plants and includes actual photographs of plants showing variations in leaves, flowers, and size and data on DNA fingerprints. Thus, a simplified selection process would be available to plant

breeders and commercial plant exporters which would facilitate mass propagation within a shorter growth period of desired variant plants.

Key words: AFLP, PCR, ornamentals, radiation, DNA fingerprinting

48. MITOCHONDRAL DNA (mtDNA) POLYMORPHISM IN THE ASIAN HONEYBEE, Apis cerana F. IN THE PHILIPPINES

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Mitochondrial nucleotide sequence was used to characterize the intergenic region between cytochrome oxidase I (COI) and cytochrome oxidase II (COII) in the motochondrial DNA (mtDNA) of the honeybee *Apis cerana F.* from four geographically isolated islands in the Philippines (Palawan, Luzon Highland and Lowland, Visayas, and Mindanao). Sequencing data revealed a 99 base pair non-coding region predominantly composed of adenine (A) and thymine (T). It is characterized by a "stem" sequence where no sequence variation was observed but had 18 variables sites found within the segment generating eight (8) haplotypes. The mitochondrial gene tree based on these haplotypes had a branch with Luzon and two of the Visayas and Mindanao haplotypes and another branch with the Palawan together with the rest of the Visayas and Mindanao haplotypes. The 3' end of the leucine+RNA gene was also sequenced but the 29 base pair segment was too short to yield enough informative characters to differentiate between haplotypes.

The mtDNA sequences of the Philippine honeybees were also compared to known mtDNA sequences of *Apis cerana* F. from Borneo, Sulawesi, Java, and Sangihe mtDNA sequences. The mitochondrial gene tree obtained showed a branch supporting Palawan haplotype together with Borneo and Java, as well as the Ozamis and Cebu haplotype from the Visayas and Mindanao group. While the Luzon haplotypes were on the other branch with the rest of the Visayas and Mindanao haplotypes. Migration of bees during the early geologic history of the Philippines may have contributed to the gene flow, thus the shared or closely related haplotypes.

Mitochondrial genome polymorphism exists among the Philippine *Apis cerana* F. The sequence diversity supports morphometric studies on Philippine honeybees showing the distinct separation of the Palawan populations but could not, how-

ever, delineate between the Luzon Lowland and Highland region. Furthermore, it showed enormous diversity among the populations from the Visayas and Mindanao regions.

Key words: *Apis cerana* F., honeybees, mitochondreal DNA (mtDNA), intergenic region, cytochrome oxidase I and II, mtDNA sequence, polyacrylamide gel electrophoresis, haplotypes, mitochondrial gene tree, genome polymorphism

49. MECHANISM OF DNA IMMUNIZATION: HOW DNA VACCINES INITIATE IMMUNE RESPONSES

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The purpose of this study is to elucidate the mechanism by which DNA immunization initiates immune responses. We found that the site of DNA inoculation (target site) played different roles in gene gun and intramuscular (IM) immunization of mice. The skin target site (following gene gun), but not the muscle, played a central, but not completely essential, role in initiating antibody and cytotoxic T lymphocyte (CTL) responses. These results indicate that the skin target site, which is rich in bone-marrow derived cells, plays bigger a role than the relatively immune-privileged muscle target site in DNA immunization. However, for both methods of immunization, CTL responses were restricted to antigen presentation by bone marrow-derived cells, not by skin or muscle cells. Furthermore, we found that antigen secretion, which presumably would lead to increased antigen migration by lymphoid tissues and increased antigen uptake by antigen-presenting cells, thus leading to increased antigen presentation in the context of MCH class II, did not enhance antibody responses. We propose a model for the mechanism of initiation of immune responses by DNA immunization based on these results and taking them within the context of results from other investigators in the field. We propose that DNA immunization initiates immune responses primarily by the direct transfection of bone marrow-derived cells that migrate rapidly out of the target site into lymphoid tissues, and that antigen expression by skin cells may be involved in raising maximal responses.

Key words: DNA immunizatin, DNA vaccines, antigen-presenting cells, antigen secretion, immune response

50. SEQUENCE ANALYSIS OF DNA VACCINE CONSTRUCTS: DETERMINING POTENTIAL RISK FOR HOMOLOGOUS RECOMBINATION WITH THE HUMAN GENOME AND OPTIMIZING CODON USAGE

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DNA immunization is a novel and highly effective means of immunization whereby plasmid DNA (DNA vaccines) encoding for antigens are delivered directly into animals or patients whose cells will then express the antigen. DNA immunization comes with the potential risk of the DNA vaccine integrating into the human genome by homologous recombination, thus possibly causing mutations that may lead to carcinogenesis. In addition, the use of gene sequences from pathogens distantly elated to humans, e.g., malaria, may affect antigen expression in the human vaccinee.

This study is designed to analyze, using the BLAST family of programs, the DNA sequences of multi-epitope DNA vaccines against dengue and malaria constructed in our laboratory. The possibility of integration of the various DNA vaccines with the human genome was examined by searching for homologies between the DNA vaccines and known human genome sequences. In addition, the sequences were analyzed with respect to the mouse genome, since the animal model used in our lab is the mouse. We have extended these studies to a DNA vaccine for hog cholera, to be used in swine, to determine whether this veterinary vaccine may integrate with the pig genome. Studies will also be conducted to detect actual integration of DNA vaccines into the genomes of recipient mice.

Finally, we analyzed the sequences used for the malaria DNA vaccines and found that some of the malaria codons in our constructs are rarely used in humans and may therefore hamper efficient antigen expression in human vaccines. Malaria DNA vaccine constructs are now being designed with optimized human codon usage, which will hopefully enhance the expression, and thus the antigenicity, of these vaccines.

Key words: DNA vaccines, sequence analysis, safety, homologous recombination, codon usage

51. USE OF RIBOTYPING AND RAMDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TO DIFFERENTIATE STRAINS OF Burkholderia andropogonis

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Burkholderia andropogonis causes leaf spots, streaks, and stripes on a wide variety of host plants such as corn, coffee, chick pea, and velvet bean. The strains of *B.* andropogonis are highly similar in phenotypic traits such as cultural, morphological, and physiological characteristics. However, the host range of B. andropogonis is exceptionally wide and diverse and *B. andropogonis* has wide geographical distribution. Some workers had attempted to group strains of *B. andropogonis* from different host plants based on pathogenicity and serological properties. However, the observed differences in host specificity were insufficient to warrant establishment of pathovars and no further work has been reported to support establishment of serovars. Knowing the relationship among strains of *B. andropogonis* will help to identify outbreaks, to determine its mode of acquisition among strains of *B. andropogonis* will help to identify outbreaks, to determine its mode of acquisition, and to define preventive measures. Unlike phenotypic properties which are not reliable for strain differentiation due to their low reproducibility and inherent variability, genotypic traits are not affected by the physiological state of the organism. In this study, genotypic methods such as ribotyping and random-amplified polymorphihc DNA (RAPD) were used to determine the relationship among 29 strains of *B. andropogonis*. In ribotyping, the chromosomal DNA was digested with either Sal I, Pst I, or Xho I and probed by digoxigen-labeled 16S rDNA of *B. andropogonis*. Hybrids were detected by chemiluminescence. In RAPD, each of the seven commercially available primers was used in low stringency polymerase chain reaction (annealing at 37°C). The amplification products were electrophoresed on agarose gel. The difference between the pattern generated by ribotyping and RAPD was established visually on the presence or absence of one or several bands. Similarity coefficients for pairwise combination were determined by Dice coefficient and clustered by the unweighted pair group method with arithmetic mean (UPGMA) procedure. All cumputations were performed using the NTSYS-PC program. Numerical analysis of the ribnpatterns generated by *Sal I, Pst* I, and *Xho* I produced a phenogram where the strains were divided into 11 clusters at a similarity of 90%. In addition, the presence of *rrn* operons

was revealed in *B. andropogonis*. RAPD analysis with seven primers grouped the strains into 12 clusters at a similarly of 90%. Comparison of the phenograms generated by ribotyping and RAPD revealed that the clusters of strains at 90% similarity were similar for these two methods. However, the relationship between clusters varied between ribotyping and RAPD, producing phenograms with different groups of clusters and different overall structure between these two methods. There was no strict correlation between the clusters and the time when the strains were isolated or the geographical origin of the isolates; between the clusters and original host; and between the clusters and pathogenicity of the strains.

Key words: *Burkholeria andropogonis*, ribotyping, RAPD, genotypic methods

52. CLONING AND CHARACTERIZATION OF THE ACYL CARRIER PROTEIN (ACP) GENE OF THE COCONUT (*Cocos nucifera* L.) ENDOSPERM

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Acyl carrier protein (ACP) is an essential cofactor in the synthesis of fatty acids. We report here the isolation and characterization of the ACP gene from coconut. The coconut endosperm ACP gene was isolated by RT-PCR using degenerate oligonucleotide primers designed specifically for the conserved region of multiply aligned ACP gene sequences from other plant species. The ~200 bp PCR product generated was cloned into a vector. DNA-sequence analysis and Southern and Northern blot analyses were subsequently performed. The results and future prospects for the cloned coconut seed ACP gene are also discussed.

Key words: acyl carrier protein, ACP, cloning, RT-PCR, Southern Blot, Northern Blot, DNA sequence, primer design

53. THE COCONUT GENE PROJECT: PRIMER DESIGN

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The general objective of the coconut gene project is to modify the fatty acid composition of coconut oil by genetic engineering. Genes for key enzymes in the lipid metabolism of coconut will be cloned and sequenced. Final expression of these genes in the coconut will be dependent on successful tissue culture methods already being worked out by the Philippine Coconut Authority (PCA).

Accomplishments so far include outlined steps and procedures that one may following designing PCR primers. Both the internet and a software called Vector NTI have been utilized in PCR primer design, a necessary start point in the said project. General guidelines for PCR primer design are hereby discussed.

Key words: vector NTI suite, primer design, lipid metabolism, Polymerase Chain Reaction (PCR), internet, annealing temperature *(Tm)*, % GC, entropy, enthalpy, free energy