

# 1<sup>st</sup> Molecular Biology & Biotechnology Symposium

**UPLB Graduate School**

*in cooperation with the*

**SEARCA Biotechnology Information Center (BIC)**

*and the*

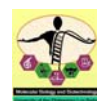
**Philippine Council for the Advancement of Science and  
Technology Research and Development  
(PCASTRD)**

SEARCA Auditorium  
January 23, 2003



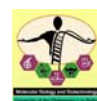
## **PROGRAMME**

1:00-1:15	Registration
1:15-1:20	Invocation <i>(Medino Gedeon Yebron, Jr.)</i>
	National Anthem <i>(Genevieve Aquino)</i>
1:20-1:30	Welcome Remarks <i>Dr. Rita P. Laude</i> <i>(Dean, UPLB Graduate School)</i>
1:30-1:50	Introduction of Keynote Speaker Keynote Address <i>Dr. Evelyn Mae T. Mendoza</i> <i>(Research Professor,</i> <i>Institute of Plant Breeding)</i>
<b>PART I</b>	
1:50-2:10	MBB Graduate Program at UPLB <i>Dr. Ma. Jamela R. Revilleza</i> <i>(Head, MBB Program Management</i> <i>Committee)</i>
2:10-2:30	Cloning the Thioesterase Gene in Coconut <i>Marni E. Cueno</i>
2:30-2:50	Genetic Analysis of Bacterial Blight Resistance in Elite Lines of Rice <i>Ma. Gay C. Carillo</i>
2:50-3:10	Isolation and Molecular Cloning of the Cocosin Promoter from Coconut ( <i>Cocos</i> <i>nucifera L.</i> ) <i>Jorge Gil C. Angeles</i>
3:10-3:25	<b>OPEN FORUM</b>
3:25-3:40	SNACKS
<b>PART II</b>	
3:40-3:55	Molecular Studies on White Fly Transmitted Gemini Viruses of Tomato <i>Dennis B. Bela-ong</i>



3:55-4:10	Molecular Analysis of Resveratrol Synthase Gene(s) in Peanut ( <i>Arachis hypogaea</i> L.) <i>Aileen N. Bayot</i>
4:10-4:25	Genetic Diversity and DNA Fingerprinting of Philippine Papayas using Minisatellite Markers <i>Maribel M. Zaporteza</i>
4:25-4:40	Isolation, purification and characterization of <i>Aspergillus</i> sp. BIOTECH 3104 and ISO 1 <i>Mary Grace B. Dacuma</i>
4:40-4:55	Establishing the Role of 14-3-3 Proteins in Rice Plant Defense <i>Neil H. Tan Gana</i>
4:55-5:10	Microbial Production of Indole-3-Acetaldehyde (IAA) <i>Dinah Q. Difuntorum</i>
5:10-5:25	<b>OPEN FORUM</b>
5:25-5:30	Closing Remarks <i>Dr. Ma. Jamela R. Revilleza</i> (Head, MBB Program Management Committee)

**Dr. Ma. Genaleen Q. Diaz**  
**Prof. Ivan Marcelo A. Duka**  
Emcees



## ORAL PRESENTATIONS

### **Cloning the Thioesterase Gene in Coconut** **Marni E. Cueno, UPLB-IBS**

Coconut (*Cocos nucifera* L.) is the major export crop of the Philippines due to its vegetable oil which has shown to be highly marketable globally. The vegetable oil of coconut is rich in medium-chain fatty acids produced primarily by the acyl-ACP thioesterase gene.

Three cloning strategies were employed to clone and isolate the putative thioesterase gene from coconut. Strategy 1 involves the use of THIO 1 and THIO 2 primers to produce a partial length of the thioesterase gene. A conserved amino acid region (DRFPDW) was observed when BLASTx and translation of the raw sequence data was performed. Comparison of the partial sequence of coconut with other plant thioesterases through a phylogenetic tree revealed a closer similarity between *Brassica napus* and that of coconut.

Strategy 2 made use of I-THIO-NQHVN-S and I-THIO-NQHVN-AS primers designed from the highly conserved amino acid region NQHVNN. Using I-THIO-NQHVN-S for 3' RACE, a 460 bp PCR band was produced. Using I-THIO-NQHVN-AS for 5' RACE, no bands were produced. When used for 3'RACE cDNA, I-THIO-NQHVN-AS produced a putative enolase gene.

Strategy 3 made use of the same PCR primer I-THIO-NQHVN-S and a gene specific primer 3' end library to probe for the putative thioesterase gene from the cDNA library. The pDNA of clones 47 and 48 revealed a similar 460 bp fragment generated by 3' RAACE suggesting that p47 and p48 contains the full-length sequence of the thioesterase gene.

### **Molecular and Phenotypic Characterization of Bacterial Blight Resistance of IRRI Elite Lines and New Plant Types** **Ma. Gay C. Carillo, IRRI**

The most widespread and destructive bacterial disease of irrigated and rainfed rice is bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The most economic and effective approach to manage BB is the use of resistant cultivars. Of the 26 BB resistance genes (*Xa*) that have been identified, the *Xa4* gene has been incorporated in most modern rice varieties. Evaluation of resistance to BB at IRRI's breeding program utilized six known races of *Xoo*



depending on screening site. At present, 12 strains representing 10 races of *Xoo* have been characterized. Five known *Xa* genes have been tagged with molecular markers and widely used for marker-assisted selection of resistant lines. BB pyramid lines containing *Xa* genes have already been used as donors to introgress *Xa* genes to elite lines as well as to the new plant type (NPT) of rice. Due to the diversity of germplasm used in breeding for various traits, the resistance to BB of 322 classical elite lines, 44 IR and PSBRc varieties was analyzed using molecular and phenotypic approaches. The effect of donor cultivars on the resistance of NPT lines was also determined and compared with lines containing tropical japonica and japonica-indica derivative lines. PCR-based markers linked to *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* were used to detect the presence of these genes on all lines. Twelve strains of *Xoo* were used to evaluate their disease reactions.

The *Xa4* gene was detected in most of the elite lines either singly or in combination with *xa5* and to an unidentified gene conferring resistance to race 9a. Most of these varieties have short lesions against races 1, 5, 7, and 10. *Xa7* was also detected in two elite lines. Detection of these genes were confirmed by their reaction to *Xoo* and compared to near-isogenic lines containing *Xa* genes. Pedigree analyses using the International Crop Information System (ICIS) showed that donors of the *Xa* genes could be TKM6, IRBB7 and IR1545-339 for *Xa4*, *Xa7* and *xa5* respectively. Seven elite lines were detected to have *Xa21* in combination with either *Xa4* or *xa5*. These lines were derived from *Oryza barthii* and either TKM6 or IR1545-339. IR5, previously characterized as susceptible to BB, showed resistance to race 9a. PSBRC82 showed high level of resistance to all races of *Xoo* except race 6 due to the presence of *Xa4* and *xa5*.

Molecular analysis of 84 NPT lines and 17 NPT donor varieties detected the presence of the *Xa4*, *xa5*, *Xa7* and *xa13* genes although some lines with gene combinations *Xa4*, *Xa4/Xa7* and *xa5/Xa7* showed susceptible reactions to all races except races 3b and 9a. Two lines carry four-gene combinations *Xa4/xa5/Xa7/xa13* that were derived from the tropical japonica background varieties, and they have very short lesions (0.9-4.5 cm) against all races of *Xoo*. Seven NPT donor varieties exhibited adult resistance to BB indicative of the *Xa3* gene suggesting that *Xa3* may also be present in the NPTs. Development of molecular markers for tagging *Xa3* is now underway.



**Isolation and Molecular Cloning of the Cocosin Promoter from Coconut  
(*Cocos nucifera* L.)  
Jorge Gil C. Angeles-UPLB-IPB**

Cocosin is a major storage protein in the coconut endosperm. Expression of the cocosin gene is tissue-specific and developmentally-regulated since it is possibly driven by a very strong promoter.

Multiple alignment analysis generated from the amino acid sequences of 11S globulins and their homologues showed the consensus regions of the protein. Based on these conserved regions, gene-specific primers were designed via backtranslation and weighed against the published coconut codon preference table. Verification using analysis of PCR products obtained from genomic DNA using web-based applications and through cDNA screening did not reveal homology towards the 11S globulins and other homologous proteins. *In-silico* verification of these consensus oligopeptides and the DNA sequences of the designed primers based on these oligopeptides showed significant homology towards 11S globulins and other related proteins.

To isolate and clone the endosperm-specific promoter from coconut, genomic DNA extracted from the coconut was used as template to generate PCR products whose DNA sequences represent the partial 5' end DNA sequence of the cocosin gene and some regulatory regions upstream. The primers used are based on the highly conserved domain near the N-terminus of the acidic chain of the cocosin protein and the reported conserved domain found in other 11S globulin promoters. The PCR products were cloned in cloning vectors and partially characterized by sequence analysis.

Sequence analysis of the plasmid DNA of two bacterial clones reveal putative promoter sequences having the reported conserved region found in other 11S globulins, putative TATA boxes and the one of the earlier reported conserved oligopeptide found after the initial ATG transcription codon. Moreover, the plasmid DNA sequence of one clone revealed the oligopeptide corresponding to the N-terminal sequence of the acidic chain of the cocosin protein (Garcia *et al*, 2001).



**Molecular Studies on Whitefly-Transmitted Geminiviruses (Geminiviridae: *Begomovirus*) in the Philippines**  
**Dennis B. Bela-ong, UPLB-IBS**

Whitefly-transmitted geminiviruses (Geminiviridae; *Begomovirus*) are serious pathogens of a large number of important crop plants in tropical and subtropical areas worldwide. Many of the diseases that they cause are among the world's most economically significant diseases, resulting to yield losses ranging from negligible to 100%, amounting to more than US\$7 billion in value of food and export crops. In the Philippines, leaf curl disease of tomato is one of the most prevalent virus diseases particularly during summer and has recently become rampant whether in the field or screenhouses of tomato growers in Laguna, Batangas, Cavite, Bulacan, and Nueva Ecija.

To provide more efficient crop protection strategies, proper identification of the viruses and a better understanding of the virus strains involved and their geographical distribution is needed. This is particularly significant for WTGs which exhibit unusual heterogeneity of nucleotide sequences among isolates from different countries. The tools of molecular biology and biotechnology/genetic engineering offer novel and more precise ways for analyzing organisms because of their specificity and sensitivity, as well as an approach to obtaining non-conventional resistance for the control of virus diseases.

This study was conducted to amplify genes of whitefly-transmitted geminiviruses (WTGs; *Begomovirus*) in the Philippines by the polymerase chain reaction (PCR), confirm the identity of the amplified DNA fragments by Southern hybridization, and to determine the presence of variability in the amplified genes through the analysis of PCR-generated restriction fragment length polymorphism (RFLP).

Leaf curl samples of tomato samples were obtained from different areas of the country and each sample was labeled as a collection. *Begomovirus* genes were amplified by the polymerase chain reaction (PCR) from total DNA extracts of different leaf curl samples using three sets of degenerate primers that amplify different regions of the DNA-A component of WTGs. Not all samples produced amplicons/PCR products of the expected sizes in PCR. Some samples produced bands in all three primer sets, some with only two of the three sets of primers, while some produced PCR fragments with only one of the three primer pairs, which suggests the presence of variation in the viral DNA sequence where the primers would anneal.

Southern blot analysis using as probe the top half clone of the DNA-A of the Philippine isolate of Tomato leaf curl geminivirus (TYLCV-Ph) confirmed the *Begomovirus* identity of the top half primer pair-generated amplicons. Restriction



enzyme analysis of the top half PCR fragments with five different restriction endonucleases (*AluI*, *HhaI*, *HpaII*, *HindIII*, and *Sau3AI*) revealed different banding profiles of agarose gel-resolved DNA digests. Total size of the cut fragments exceed that of the original uncut fragment which suggests the presence of more than a single *Begomovirus* isolate in a sample, which have not been discriminated by PCR. In some instances, the presence of uncut fragments was observed and this could be attributed to incomplete enzyme digestion or it could represent a genotype that lacks the restriction site for a particular endonuclease. The presence of polymorphism suggests variability among the different *Begomovirus* isolates studied.

### **Molecular Analysis of Resveratrol Synthase Gene(s) in Peanut (*Arachis hypogaea* L.)**

**Aileen N. Bayot, UPLB-IPB**

*(note: as of 2006, she is affiliated with UPLB-BIOTECH)*

Resveratrol (3,4', 5 trihydroxystilbene) belongs to one specific class of phenolics called the stilbenes. These widely distributed phenolics have been isolated from diverse plant families including trees such as eucalyptus, spruce, Scotts pine, and even in a few flowering plants such as the lily. Grapes are a particularly good source of resveratrol. It occurs in the vines, roots, seeds, and stalks, but its highest concentration is in the skin (50-100 µg per gram). On the other hand, a fluid ounce of red wine averages 160 µg of resveratrol, as compared to peanuts, which averages 73 µg per ounce.

The constitutive accumulation of stilbenes is believed to act as a general mechanism in anti-microbial disease resistance. In the case of resveratrol, its accumulation acts as a phytoalexin, a low molecular weight anti-microbial compound that accumulates in plants as a result to infection or stress. In addition, resveratrol has been believed to play a significant role in the pharmacological activity of medicinal plants through antioxidant or chemopreventive activities. Resveratrol has also been shown to inhibit platelet aggregation, protect the liver from lipid peroxidation and inhibit low-density lipoprotein oxidation. Because of resveratrol's potential as a phytoalexin and its pharmacological properties, there is increasing desire to incorporate it into crops that cannot produce this compound. Because of this, its pathway was studied and elucidated.

The initial step in the biosynthesis of resveratrol is through the condensation of one molecule of *p*-coumaroyl CoA and three molecules of malonyl CoA by resveratrol synthase (RS). Chalcone synthase (CHS), an enzyme utilizing the same substrates but synthesizing a different product



(naringenin chalcone) is closely related to RS. The comparative analysis of cloned sequences revealed that the two enzymes can be clearly distinguished by characteristic differences in amino acid sequences that presumably reflect the different product specificities.

Resveratrol synthase is potentially an interesting enzyme for genetic engineering because it synthesizes a phytoalexin in one step from precursors available in all plants and because the gene is absent in most of the important crop plants. In fact, it has already been transformed in crops such as tobacco, rice, barley, wheat, tomato and alfalfa. These reports demonstrate that the expression of an RS gene in a foreign plant species may offer a broad spectrum of increased resistance to fungal pathogens.

In this study, the resveratrol synthase gene(s), will be isolated and cloned using PCR. The isolated gene(s) will then be compared with known RS sequences to verify their identity and to determine if there is considerable difference between other RS genes from peanut.

### **Genetic Diversity and DNA Fingerprinting of Philippine Papayas using Minisatellite Markers**

***Maribel M.Zaporteza, UPLB-IPB***

Very little is known on the extent of genetic diversity among the Philippine papayas and their wild progenitors. Hence, this study aims to provide basic information on their genetic base in order to improve the efficiency of the papaya improvement breeding program. Minisatellite markers or variable number tandem repeats (VNTRs) are DNA markers that provide high level of certainty in genotype identification.

VNTRs were obtained from the papaya sequences in the Genbank database and 9 primers were designed based on their highly conserved flanking regions. A polymerase chain reaction (PCR) protocol was developed using these primers to amplify the minisatellites. The PCR products were electrophoresed in a 3% agarose gel, stained with ethidium bromide and visualized under UV light.

Initial efforts using three primer pairs generated SSRs from 40 papaya lines. A high level of polymorphism was observed demonstrating the minisatellites capability to quantify genetic diversity and identify the different papaya genotypes.



## **Isolation, Purification and Characterization of Glucoamylase from**

*Aspergillus* sp. **BIOTECH 3104 and ISO 1**

**Mary Grace B. Dacuma, UPLB-BIOTECH**

*(note: as of 2006, she is affiliated with UPLB- IBS)*

Glucoamylase was produced from fungal and bacterial isolates *Aspergillus* sp. BIOTECH 3104 and ISO 1, respectively. The fungal and bacterial glucoamylases were purified by differential centrifugation to separate the soluble enzyme from the biomass, ultrafiltration, ammonium sulfate precipitation, dialysis, ion exchange chromatography using DEAE-cellulose and gel filtration chromatography using Sephadex G-100. The enzymes were assayed for volumetric activity, protein content and specific activity at each step of purification. The molecular weights of the purified glucoamylases were determined by SDS-PAGE. The effects of different pH, different denaturing agents (2% SDS, 0.8 M urea and 2 M guanidium chloride) and inhibitors (1 mM EDTA and metal ions), as well as the effect of the incubation temperature on the volumetric activity of the enzymes were studied. The effect of the storage time on shelf life was also determined for four consecutive weeks using 1% sodium benzoate and 1% potassium sorbate as preservatives.

## **Establishing the Role of 14-3-3 Proteins in Rice Plant Defense**

**Neil H. Tan Gana, UPLB-IPB**

The 14-3-3 proteins play important roles in regulating signal transduction mechanisms (Shenke et al, 2002), and in plant defense responses (Collinge et al, 2002). Using bioinformatics analyses of the rice genome targeting 14-3-3 candidate genes, in tandem with Targeting Induced Local Lesions In Genomes (TILLING) technique, selected IR64 rice mutants will be subjected to link one or more 14-3-3 gene loci with plant defense function. This research activity will also contribute towards the further design and implementation of a rice mutant database system at the International Rice Research Institute (IRRI).



**Microbial Production of Indole-3-Acetaldehyde (IAA)**  
***Dinah Q. Difuntorum, UPLB-BIOTECH***

The growth of plants is controlled by different plant growth regulators (PGRs). PGRs are natural or synthetic organic compounds, other than nutrients, which in small amounts promote or inhibit or otherwise modify any physiological process in plants. Of the different plant hormones, auxins are of special interest because this group plays important roles in the coordination of plant growth and development. Auxin promotes the growth of vascular tissue and vascular cambium. It increases fruit growth and prevents premature falling off of leaves, fruits or flowers. Auxin is the active ingredient in most rooting compounds in which cutting are dipped during vegetative propagation. However, chemically synthesized auxins used as plant growth regulators are mostly imported; hence, expensive. Therefore, other sources of this phytohormone should be explored.

Indole-3-acetic acid (IAA) is one of the most abundant naturally-occurring and physiologically active auxin. It is involved in regulating a variety of developmental and cellular processes such as cell extension, cell division, vascular differentiation, root formation, apical dominance and tropisms. About 80% of bacteria can produce IAA identical to that found in plants. These include root-associated rhizobacteria termed plant growth-promoting rhizobacteria (PGPR). PGPR can then be used as factories for the production of PGRs.

PGPR Isolates which exhibited growth promotion of vegetable crops under greenhouse conditions were screened for IAA production. The highest IAA-producers were characterized and identified. The probable biosynthetic pathway for IAA production has been identified in selected isolates. Afterwards, the specific gene for the production of IAA will be isolated and sequenced. Molecular cloning will be done to produce a recombinant bacteria which could overproduce auxin.

