Professor Dr. Victor I. Mocanu
Department of Geophysics, Faculty of Geology and Geophysics,
University of Bucharest, 6 Traian Vuia Street, RO-020956
Bucharest 2, ROMANIA

Interactions of Earth's Surface and Deep Process in Active Geodynamic Area of High Natural Hazard

Southeast Europe represents a key natural laboratory for research on the quantification of the neotectonic processes, development and validation of a new generation of models for the ongoing orogeny and its obvious effect on the dynamic topography. The Pannonian-Carpathian system represents an area of significant natural hazard risk in Europe where, in addition to landslides, flooding and earthquakes, human activities contribute to stress the complex environment. The currently running research is process-oriented. It can improve the understanding of what determines the present day situation and generates active changes in the sedimentary input into the basins and thus affecting the entyre regional dynamics of the upper globe. An integrated and strategic management of interdisciplinary research activities in the field of Earth Sciences designed and carried out in a synergetic way is needed for the mitigation of the highest natural hazard in Southeast Europe.

Inherited orogenic patterns probably control the distribution of neotectonic activity and the location/dynamics of the natural hazards of all types. The final stages of continental collision are characterized by frequent episodes of structural inversions, leading to vertical movements at regional and local scales. This is associated with significant changes in recent lithosphere dynamics, tectonic topography, landscape evolution, and climate inversions.

Southeast Europe provides one of the best natural laboratories in the world for the regional-scale integrated assessment of an entire orogenic back-arc system, addressing interactions between active mountain uplift processes, through drainage networks to active on and offshore basins. The incorporation of long-term and deep lithospheric processes into a study of natural hazards represents a major challenge for geosciences. Quantifying the links between lithosphere dynamics, neotectonics and surface and climate processes is a key target through the development of numerical models, making use of data sets that cover different aspects of these phenomena at a wide range of scales.

Professor Dr. Tom Corcoran
Co-Director of the Consortium for Policy Research in Education (CPRE),
University of Pensylvania, USA

Recent Research Findings on Science Teaching and Learning in the United States, and the Implications for Thailand

This presentation will address three important questions about science education in the United States:

- Are the current science standards and the typical K-8 curriculum moving us in the right direction?
- Are our current reform strategies producing significant improvements in the teaching and learning of science? If not, why not?
- What kinds of reforms might produce more rapid progress in science education?

And then address the question of the implications of the answers to these questions for Thailand.

The presentation will draw heavily on data from four major studies: the national evaluation of the Local Systemic Change program supported by the National Science Foundation; a study of how teachers think about their practice supported by the Pew Charitable Trusts; a study led by the presenter of improvement efforts in Texas; and the new study of K-8 science produced by the National Research Council.

The presenter will draw on these and related studies conducted in the US to outline a strategy for improving science teaching and learning in the US; and then challenge the audience to consider the implications of these findings for reforms in science standards, curriculum, professional development, and instructional support in Thailand.

Professor Dr. Graham N. George
Canadian Light Source, University of Saskatchewan, CANADA

Uses of Synchrotron Light in Agriculture, Biology, Chemistry, Environment and Other Sciences

X-ray absorption spectroscopy (XAS) using synchrotron light provides a powerful probe of both physical and electronic structure. As spectroscopic methods go it is a relatively new technique, originating in the early 1970's, and in recent years it has become increasingly applied to a very wide variety of fields. X-ray absorption spectra arise from core-level excitation by absorption of X-rays, and are thus associated with an absorption edge (e.g. 1s excitation for a K-edge). They are usually separated into two different regions: the Extended X-ray Absorption Fine Structure or EXAFS, which occurs at energies higher than the absorption edge; and the near-edge region which consists of features before the major inflection, and any after the inflection which are not part of the analyzable EXAFS. XAS is element specific and can be used to investigate solids, liquids (including solutions), gaseous materials, and any mixtures thereof. It probes all of an element within a sample with moderate sensitivity, and is applicable to a very wide range of elements. XAS theory is well developed, and the methods of analysis of the EXAFS part of the spectrum to give a local radial structure for the absorbing atom, are also well established.

The scientific areas in which XAS has been employed are very diverse; areas of application encompass the biological, chemical, physical and many other branches of sciences. As a complete review of all applications in these fields is impossible, Prof. Graham George will illustrate the use of the technique by example and will focus on examples from his own research. In general there is some overlap between fields, for example many biological studies have a strong chemical component, and all depend upon the physics of X-ray absorption.

Professor Dr. Helmut Wiedemann
National Synchrotron Research Center (NSRC), THAILAND

X-rays at the SIAM Photon Laboratory

The SIAM Photon Source is designed to produce an intense beam of synchrotron radiation in a spectral range spanning from the Infra-red to hard X-rays. This radiation can be used for basic and applied research in many areas ranging from Agriculture, Biology, Chemistry, Drug Development, Environmental studies, Material Science to Physics and other areas. The wide application of this radiation is due to its short wavelength, especially that of X-raysbeing of the order of 1 Angstroem, which is the scale for atomic arrangments and is therefore especially suited to study atomic and molecular structures. The high intensity of the radiation allows the detection and study of minute sample concentrations, the determinatiuon of atromic structures, it can be used for microscopy with a resolution below 50 nm. Scattering synchrotron radiation from crystals is widely used to study and solve protein structures in support of drug developments. Environmental science makes use of synchrotron radiation because of its high sensitivity and element specifity.

In this talk, experimental capabilities and opportunities at the SIAM Photon Source will be discussed together with examples of applications at worldwide laboratories and potential applications of interest in Thailand and surrounding countries.

The 2004 Outstanding Government Officer Awards and
The 2006 L'Oreal For Women in Science Research Scholar

Associate Professor Dr. Supa Hannongbua
Department of Chemistry, Faculty of Science, Kasetsart University,
Bangkok 10900, THAILAND

Recent Science and Technology of Thai Herbs Research and Funding in Thailand

Thailand as a Tropical country has been rich with the variety of medicinal herbs. Thai people have made use of these herbs for their traditional ways of living for millennia. These herbs also provide novel materials for development of modern biotechnology. A basic knowledge of these is needed in order to use and develop biological resources in a sustainable manner. Therefore, understanding of the current science and technology of Thai herbs research and source of funding in Thailand will be focused.

เสวนาพิเศษ "ตามรอยสัตว์โบราณ"

ดร. วราวุธ สุธีธร
สำนักธรณีวิทยา กรมทรัพยากรธรณี กระทรวงทรัพยากรธรรมชาติและสิ่งแวดล้อม
ถนนพระรามที่ 6 ราชเทวี กรุงเทพฯ 10400

ภาพรวมของการศึกษาของโครงการ: ไขความลับจากฟันของไดโนเสาร์ยักษ์ใหญ่

ไดโนเสาร์ซอโรพอด เป็นไดโนเสาร์กินพืชขนาดใหญ่ คอยาว หางยาว และเดิน 4 ขา ซอโรพอดในแผ่นดินไทยพบค่อนข้างหนาแน่นในเขตที่ราบสูงโคราช และพบบ้างบริเวณภาคตะวันออก ภาคเหนือและภาคใต้ของประเทศ กระดูกไดโนเสาร์ที่พบมีอายุต่างกัน เพราะอยู่ในชั้นหินต่างกัน อย่างน้อย 6 หมวดหิน มีเพียง 2 ชนิดที่สามารถวิจัยจนจำแนกชนิดได้ และเป็นชนิดใหม่ด้วย ได้แก่ *อิสานโนซอรัส อรรถวิภัชน์ชิ* จากหมวดหินน้ำพอง อยู่ในช่วงยุคไทรแอสสิกตอนปลาย และเป็นหนึ่งในบรรพบุรุษของซอโรพอดที่เก่าแก่ที่สุดในโลก และอีกชนิดหนึ่ง คือ *ภูเวียงโกซอรัส สิรินธรเน่* จากหมวดหินเสาขัว อยู่ในช่วงยุคครีเตเชียสตอนต้น เป็นซอโรพอดที่พบมากที่สุดในประเทศไทย มีการค้นพบในหลายจังหวัด เช่น ขอนแก่น กาฬสินธุ์ หนองบัวลำภู โดยเฉพาะที่แหล่งภูกุ้มข้าว (กาฬสินธุ์) มีกระดูกของ ภูเวียงโกซอรัส มากองรวมกันอยู่มากกว่า 500 ชิ้น และมีความสมบูรณ์มากด้วย มีหลายแหล่งที่พบกระดูกเพียงไม่กี่ชิ้น ทำให้จำแนกชนิดได้ยาก อาจระบุได้แค่กลุ่มหรือวงศ์ของไดโนเสาร์เท่านั้น มีชิ้นส่วนหนึ่งที่ช่วยให้การจำแนกไดโนเสาร์ง่ายขึ้น นั่นก็คือ ฟันไดโนเสาร์ เพราะฟันเป็นกระดูกส่วนที่แข็งที่สุด และมีความจำเพาะของไดโนเสาร์แต่ละชนิด จากลักษณะของฟันซอโรพอดที่พบในชั้นหินกลุ่มโคราช สามารถแบ่งเป็น 2 กลุ่มใหญ่ คือ กลุ่มที่มีฟันรูปช้อน และ กลุ่มที่มีฟันแบบแท่งดินสอ

ฟันรูปช้อน เป็นลักษณะของซอโรพอดวงศ์ยูฮิลโลโพดิเด่ อยู่ในยุคจูแรสสิกตอนปลายของหมวดหินภูกระดึง ในช่วงยุคครีเตเชียสตอนต้นของหมวดหินเสาขัว พบฟันซอโรพอดทั้ง 2 แบบ คือ ฟันแบบแท่งดินสอ ของ *ภูเวียงโกซอรัส สิรินธรเน่* ซึ่งอยู่ในกลุ่มนีเมกโตซอริด เจอร่วมกัน ฟันรูปช้อน ของซอโรพอดกลุ่มยูฮิลโลโพดิด เมื่อเข้าสู่ช่วงปลายของยุคครีเตเชียสตอนต้น ในหมวดหินโคกกรวด พบแต่ฟันแบบแท่งดินสอของซอโรพอดกลุ่มนีเมกโตซอริดเท่านั้น

เป็นไปได้ว่าเกิดการแทนที่ซอโรพอดที่มีฟันรูปช้อนโดยซอโรพอดที่มีฟันแบบแท่งดินสอในช่วงยุคครีเตเชียสตอนต้น อาจจะมีสาเหตุมาจากการเปลี่ยนแปลงของพืชพรรณในยุคนั้น และ / หรือ การแข่งขันระหว่างเผ่าพันธุ์กับไดโนเสาร์ชนิดอื่น

นางสาวศศิธร ขันสุภา และ นายสุรเวช สุธีธร
สำนักธรณีวิทยา กรมทรัพยากรธรณี กระทรวงทรัพยากรธรรมชาติและสิ่งแวดล้อม
ถนนพระรามที่ 6 ราชเทวี กรุงเทพฯ 10400

ไดโนเสาร์ออร์นิธิสเชียนในประเทศไทย

ประเทศไทยมีการค้นพบ สำรวจ และศึกษาวิจัยฟอสซิลไดโนเสาร์อย่างต่อเนื่องกว่า 25 ปีมาแล้ว พบไดโนเสาร์จำนวนมากมายทั้งไดโนเสาร์กินพืชและกินเนื้อ ในกลุ่มไดโนเสาร์กินพืชนอกจากพวกซอโรโพดา (Sauropoda) คณะสำรวจไดโนเสาร์ไทย-ฝรั่งเศสยังได้พบร่องรอยของไดโนเสาร์ออร์นิธิสเชียน (Ornithiscian dinosaur) หรือไดโนเสาร์กลุ่มที่มีกระดูกสะโพกคล้ายนก (bird-hipped dinosaur) ในชั้นหินกลุ่มหินโคราช ภาคตะวันออกเฉียงเหนือ ซึ่งสามารถจำแนกเป็นสองกลุ่ม กลุ่มแรกได้แก่ ไธรีโอโฟรา (Thyreophora หรือ armored dinosaurs) พบปรากฏเป็นครั้งแรกที่จังหวัดกาฬสินธุ์ โดยพบกระดูกสันหลังเพียงชิ้นเดียวจัดอยู่ในพวกสเตโกซอเรีย (Stegosauria) จากชั้นหินทรายหมวดหินภูกระดึง อายุจูแรสซิกตอนกลาง ไม่พบหลักฐานเพิ่มเติม และในเวลาต่อมาพบร่องรอยของไดโนเสาร์กลุ่มหลัง ได้แก่ เซอราโพดา (Cerapoda) ได้แก่ พวกเซอราทอปเซีย (Ceratopsia หรือ horned dinosaur) พบเพียงกรามบนและล่างของไดโนเสาร์ปากนกแก้ว ที่จังหวัดชัยภูมิในชั้นหินทรายหมวดหินโคกกรวด อายุครีเทเชียสตอนต้น จากการศึกษาวิจัยพบว่าเป็นไดโนเสาร์สกุลเดียวกับที่พบในจีนและเป็นชนิดใหม่ของโลก คือ ซิตตะโกซอรัส สัตยารักษ์กี (*Psittacosaurus sattayaraki*) ยังไม่พบหลักฐานอื่นเพิ่มเติมอีก นอกจากนี้พบชิ้นส่วนกระดูกไดโนเสาร์พวกออร์นิโธโพดา (Ornithopoda) ในหลายบริเวณ ได้แก่พวกฮิปซิโลโฟดอนทิด (Hypsilophodontid) พบกระดูกขาหลังท่อนบนขนาดเล็กคล้ายพวก ฮิปซิโลโฟดอน (*Hypsilophodon*) ที่จังหวัดมุกดาหาร ในชั้นหินทรายหมวดหินภูกระดึง อายุจูแรสซิกตอนกลาง ยังไม่พบหลักฐานเพิ่มเติมจากเดิม และพวก อิกัวโนดอนเทีย (Iguanodontia) พบหลายแห่งในชั้นหินทรายหมวดหินโคกกรวด อายุครีเทเชียสตอนต้น เริ่มจากจังหวัดกาฬสินธุ์ พบส่วนปลายล่างของกระดูกขาหลังท่อนบน และจังหวัดอุบลราชธานี พบหลักฐานจากฟัน กระดูกสันหลัง และกระดูกขาหลังท่อนบน หลักฐานล่าสุดที่จังหวัดนครราชสีมา พบร่องรอยของทั้งพวกอิกัวโนดอนเทีย (Igaunodontia) และพวกแฮดโดรซอริดี (Hadrosauridae หรือ duck-billed dinosaurs) โดยพบหลักฐานของกรามและฟันบางส่วน กระดูกสันหลัง กระดูกซี่โครง และเศษชิ้นส่วนกระดูกแขนและขา ยังไม่พบหลักฐานที่บ่งชี้ชัดเจน และตัวอย่างทั้งหมดอยู่ในระหว่างการศึกษาวิจัยในรายละเอียดและสำรวจหาหลักฐานเพิ่มเติม เมื่อเร็ว ๆ นี้ พบหลักฐานของรอยตีนไดโนเสาร์ออร์นิโธพอดในชั้นหินทรายหมวดหินโคกกรวด ช่วงอายุครีเทเชียสตอนต้น ที่อำเภอท่าอุเทน จังหวัดนครพนม ร่องรอยบรรพชีวินเหล่านี้เป็นหลักฐานของวิวัฒนาการของสิ่งมีชีวิตโบราณบนผืนแผ่นดินไทย และเป็นหลักฐานบ่งชี้ว่ามีการแลกเปลี่ยนระหว่างกลุ่มสิ่งมีชีวิตและการเชื่อมต่อกันของแผ่นดิน (land connection) ในช่วงตอนต้นมหายุคมีโสโซอิกของแผ่นอนุทวีปฉาน-ไทยและแผ่นอนุทวีปอินโดจีน จากหลักฐานการมีอยู่และความคล้ายคลึงกันของไดโนเสาร์พวกออร์นิธิสเชียน และไดโนเสาร์อีกหลายชนิดของไทยกับไดโนเสาร์กลุ่มเดียวกันในทวีปเอเชีย ยุโรป อเมริกา และแอฟริกา

นางสาวธิตา แสนยะมูล
สำนักธรณีวิทยา กรมทรัพยากรธรณี กระทรวงทรัพยากรธรรมชาติและสิ่งแวดล้อม
ถนนพระรามที่ 6 ราชเทวี กรุงเทพฯ 10400

รอยเท้าไดโนเสาร์ในประเทศไทย

รอยเท้าของสิ่งมีชีวิตสามารถบอกถึงกิจกรรม และพฤติกรรมของเจ้าของรอยเท้าในขณะที่มันมีชีวิตอยู่ ว่ากำลังเดิน วิ่ง หรือหาอาหาร ฟอสซิลรอยเท้าไดโนเสาร์ ประกอบด้วยสองส่วนด้วยกัน คือ ส่วนของแม่พิมพ์ (mould) และส่วนของรูปหล่อ (cast) จากลักษณะสัณฐานของรอยเท้า และแนวทางเดิน สามารถบอกเจ้าของรอยเท้าได้ ว่าเป็นพวกที่เดินด้วยสองขาหลัง หรือ เดินด้วยสี่ขา กินพืช หรือ กินเนื้อเป็นอาหาร นอกจากนี้ขนาดของรอยเท้า ระยะก้าว และระยะห่างระหว่างก้าว สามารถบอกความสูง และอัตราเร็วในการเดิน การวิ่ง ของเจ้าของรอยเท้าได้ รอยเท้าไดโนเสาร์ที่พบในประเทศไทยเคยมีการรายงานจากหมวดหินพระวิหาร อ.ภูเวียง จ.ขอนแก่น อุทยานแห่งชาติเขาใหญ่ จ.ปราจีนบุรี ภูแฝก จ.กาฬสินธุ์ ภูเก้า จ.หนองบัวลำภู และหมวดหินภูพาน เขตรักษาพันธุ์สัตว์ป่า ภูหลวง อ.ภูหลวง จ.เลย หมวดหินโคกกรวดพบฟอสซิลของสัตว์มีกระดูกสันหลังหลายชนิด แต่ฟอสซิลที่เป็นรอยเท้าสัตว์มีกระดูกสันหลังในหมวดหินนี้พบในแหล่งเดียวเท่านั้น คือที่อ.ท่าอุเทน จ.นครพนม แหล่งรอยเท้าไดโนเสาร์เป็นแหล่งที่มีความสำคัญ เนื่องจากเราสามารถศึกษาถึงรูปร่างลักษณะ การดำเนินชีวิต ของสิ่งมีชีวิตในอดีต รวมทั้งสภาพแวดล้อมในช่วงอายุนั้น เนื่องจากรอยเท้าประทับอยู่บนแผ่นหินทรายถ้าทิ้งไว้นาน ๆ จะถูกกัดเซาะสึกกร่อนได้นอกจากนี้ยังมีผู้ลักลอบนำรอยเท้าออกจากแหล่ง จึงควรจะดำเนินการอนุรักษ์ อาจมีการทำหลังคาป้องกันการสึกกร่อนจากน้ำฝน แสงแดด และทำรั้วกั้น จากนั้นก็พัฒนาให้เป็นแหล่งท่องเที่ยวทางวิชาการต่อไป

นายคมศร เลาห์ประเสริฐ
ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหาสารคาม จ. มหาสารคาม 44150

ฟอสซิลจระเข้ในที่ราบสูงโคราช มหายุคมีโซโซอิกของประเทศไทย

จากการศึกษาอย่างต่อเนื่องของทีมนักวิจัยบรรพชีวินไทย-ฝรั่งเศส พบจระเข้มหายุคมีโซโซอิกบริเวณที่ราบสูงโคราช 9 ชนิด 6 สกุล จาก 3 วงศ์ จระเข้สกุล *Theriosuchus* จากหมวดหินภูกระดึงและเสาขัวและ Teleosaurid, cf. *Peipehsuchus* จากหมวดหินภูกระดึงได้ถูกพบเป็นครั้งแรก ฟอสซิลจระเข้จากภูพอก จ.สกลนคร ในหมวดหินเสาขัวและฟอสซิลจระเข้จากบ้านสะพานหิน จ.นครราชสีมาในหมวดหินโคกกรวด ปรากฏลักษณะที่แตกต่างจากจระเข้ในวงศ์ (Family) เดียวกัน ขณะนี้อยู่ในขั้นตอนการตั้งขึ้นเป็นจระเข้ชนิดและสกุลใหม่ของโลก *Theriosuchus* sp. A จากหมวดหินเสาขัว "*Goniopholis*" sp A และ "G " sp B จากหมวดหินโคกกรวด มีลักษณะเฉพาะซึ่งเพียงพอสำหรับการตั้งขึ้นเป็นชนิดใหม่ได้ แต่ต้องได้รับการเตรียมตัวอย่างเพิ่มเติมก่อน "*Sunosuchus*" *thailandicus* Buffetaut and Ingavat, 1980 และ "*Goniopholis*" *phuwiangensis* Buffetaut and Ingavat, 1983 ได้ถูกจัดอยู่ในสกุลที่ไม่แน่นอน เนื่องจากยังขาดลักษณะที่เหมาะสมและชัดเจนในการใช้จำแนกระดับสกุลและชนิด

นางสาวอุทุมพร ดีศรี
สำนักธรณีวิทยา กรมทรัพยากรธรณี กระทรวงทรัพยากรธรรมชาติและสิ่งแวดล้อม
ถนนพระรามที่ 6 ราชเทวี กรุงเทพฯ 10400

การศึกษามอร์โฟเมทริก ปลาโลปิโดเทส พุทธบุตรเอนซิส แหล่งภูน้ำจั้น หมวดหินภูกระดึง จังหวัดกาฬสินธุ์

ตะกอนภาคพื้นทวีปช่วงมหายุคเมโสโซอิกของกลุ่มหินโคราชบริเวณภาคตะวันออกเฉียงเหนือของไทย เป็นตะกอนที่มีความหลากหลายของซากสัตว์มีกระดูกสันหลัง แต่ซากปลากระดูกแข็งยังมีการค้นพบเป็นจำนวนน้อยมาก และไม่สมบูรณ์ ส่วนใหญ่เป็นเศษฟัน เกล็ด และแผ่นฟัน ข้อมูลการค้นพบมีการเปลี่ยนแปลง หลังจากการค้นพบแหล่งปลาบริเวณภูน้ำจั้น ตำบลเหล่าใหญ่ อำเภอกุฉินารายณ์ จังหวัดกาฬสินธุ์ แหล่งปลาบริเวณภูน้ำจั้น จัดอยู่ช่วงปลายของหมวดหินภูกระดึง ปัจจุบันจัดให้เป็นหมวดหินล่างสุดของกลุ่มหินโคราช อย่างไรก็ตามการระบุอายุยังไม่แน่นอน แต่เมื่อพิจารณาจากอายุของหมวดหินที่วางตัวปิดทับอยู่ หมวดหินภูกระดึงน่าจะมีอายุอยู่ในช่วงปลายของยุคจูแรสสิก ถึงต้นยุคครีเทเชียส

ภูน้ำจั้นเป็นแหล่งปลาวงศ์ Semionotiformes ซึ่งถูกอนุรักษ์ไว้อย่างสมบูรณ์ มีการค้นพบและอธิบายตัวอย่างแล้วจำนวน 150 ตัว เป็นปลาชนิดใหม่ 1 ชนิดคือ *Lepidotes buddhabutrensis* สภาพการอนุรักษ์มีตั้งแต่เป็นเศษชิ้นส่วนของลำตัวที่แตกหักแต่มีการเชื่อมต่อของแถวเกล็ด หัวกะโหลก ไปจนถึงตัวอย่างที่เชื่อมติดกันอย่างสมบูรณ์ทั้งตัว

หลังจากทำความสะอาดด้วยเครื่องสกัด air pen แล้วพบว่าปลามีรูปร่างหลายแบบ แต่จากการศึกษามอร์โฟเมทริก พบว่าตัวอย่างปลา *L. buddhabutrensis* ทั้งหมดมาจากประชากรเดียวกันที่ตายพร้อมกันเป็นจำนวนมาก ซึ่งการปรากฏความหลากหลายทางรูปร่างของปลา *L. buddhabutrensis* อาจเกิดจากความหลากหลายของสภาพการอนุรักษ์

Assistant Professor Dr. Joongjai Panpranot
Center of Excellence on Catalysis and Catalytic Reaction Engineering, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, THAILAND

Effect of Tio_2 Crystalline Phase on the Characteristics and Catalytic Properties of Pd/Tio_2 Catalyst in Acetylene Hydrogenation

Pd/TiO_2 catalysts have been prepared using TiO_2 supports consisting of various rutile/anatase crystalline phase compositions. Increasing amount of %rutile phase in the TiO_2 resulted in a decrease in BET surface areas, lower amount of Ti^{3+} sites, and lower Pd dispersion. While acetylene conversions were found to be merely dependent on Pd dispersion, ethylene selectivity appeared to be strongly affected by the presence of Ti^{3+} in the TiO_2 samples. When TiO_2 samples with 0-44% rutile were used, high ethylene selectivities (58-93%) were obtained whereas ethylene losses occurred for those supported on TiO_2 with rutile phase 85 or 100%. XPS and ESR experiments revealed that significant amount of Ti^{3+} existed in the TiO_2 samples composed of 0-44% rutile. The presence of Ti^{3+} in contact with Pd can probably lower adsorption strength of ethylene resulting in an ethylene gain. Among the five catalysts used in this study, the results for Pd/TiO_2-R44 suggest an optimum anatase/rutile composition of the TiO_2 used to obtain high selectivity of ethylene in selective acetylene hydrogenation.

Keywords: titania polymorph, crystalline phase composition, Pd/TiO_2, selective acetylene hydrogenation

Associate Professor Dr. Shanop Shuangshoti
Department of Pathology, Faculty of Medicine,
Chulalongkorn University, Bangkok 10330, THAILAND

Research in Anatomic Pathology

Anatomic pathology is a branch of pathology, which primarily pays attention to morphological changes of the tissue or cell in a variety of conditions. Since the morphological alterations in most of the human diseases have long been recognized, the study is sometimes viewed as an outdated issue. It has also been challenged by the modern techniques, and its existent has been questioned in many aspects. Nevertheless, since most diseases are complex, a single tool or subject is, therefore, unlikely to unravel the underlying mechanisms. In this review, applications of the morphological study in today research in human diseases will be highlighted. In the personal viewpoint, knowledge in anatomic pathology is still playing a significant role in modern research. Advantages and disadvantages of the morphological study will be provided. Opportunities and limitations regarding pathology research in Thailand will also be discussed.

Assistant Professor Dr. Puangratana Pairor
School of Physics, Institute of Science, suranaree University of Technology, Nakhon Ratchasima 30000, THAILAND

Understanding Metal-Superconductor Junctions

The main objective of this talk is to give an introduction to tunneling spectroscopy of a metal-superconductor junction. I will start by reviewing a few physical properties of a metal and a superconductor, and then describe a simple approach, which I have been using to study these junctions, called the Blonder-Tinkham-Klapwijk (BTK) formalism. The advantage of this method over others will be briefly discussed. At the end, I will show some of my recent results related to the tunneling spectroscopy of d-wave superconductors.

Dr. Visith Thongboonkerd
Medical Molecular Biology Unit, Office for Research and Development,
Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Proteomics - An Important Key to Unravel Mysteries Behind Human Diseases

Despite adequate treatment with available standard regimens, therapeutic responses in some human diseases remain unsatisfactory. This may indicate that details of these diseases are still poorly understood and need to be unraveled. One of the most important molecules, which play critical roles in the pathogenic mechanisms of diseases, is protein. Recently, proteomic technologies have been emerging, making simultaneous analysis of a large number of proteins feasible. Major objectives of applications of proteomics to medicine include: (i) better understanding of normal physiology and pathophysiology of diseases; (ii) identification of novel therapeutic targets and drugs; (iii) biomarker discovery (for early diagnosis, prediction of therapeutic response and prognosis); and (iv) vaccine discovery (for successful prevention of diseases). In this session, current research projects handled by this investigator, including (a) applications of proteomics to kidney diseases; (b) applications of proteomics to non-kidney diseases; and (c) developments of new proteomic methodologies/technologies, will be highlighted.

Assistant Professor Dr. Apinpus Rujiwatra
Research Laboratory for Excellence in Nano and Smart Materials
Faculty of Science, Chiang Mai University, Chiang Mai 50200,
THAILAND

Development and Applications of "Chimie Douce" Techniques: Synthesis and Characterization of Lead Titanate (PT) *via* (Sonocatalytic) Solvothermal Synthesis

Solvothermal synthesis is one of the most pronounced "chimie douce" techniques, due to the capability in controlling over chemical stoichiometry, phasic purity, particle shape and size, for examples, of the synthesized solids affordable from mostly one-pot procedure. Relatively less cost for instrumentation, chemical precursors and energy consumption is also attractive among the materials technologists. Here, the application of the technique in the preparation of one of the ideal piezoelectric materials, i.e. lead titanate (PT), is reported as an example. The mild temperature of as low as 150°C with very short reaction time of only 3 hours can evidentially provide phase-pure PT. The influences of various synthetic parameters, e.g. type and concentration of mineralizers, reaction temperature and time, on phase purity, particle size and shape are discussed with experimental evidences. The formation mechanism of PT under the investigated conditions is also postulated. The attempts in developing the as-called "sonocatalytic solvothermal synthesis" are also reported. This has led to the improvement in lowering the reaction temperature to as low as 130°C with similar reaction time of 3 hours to give phase-pure PT with better particles characters.

ABSTRACTS OF CONTRIBUTED PAPERS
ORAL AND POSTER PRESENTATION

A_A0001 TRAVELING WAVE FRONT SOLUTIONS IN NEURAL NETWORK MODEL

Sittipong Ruktamatakul[1], Yongwimon Lenbury[2], and Jonathan Bell[3]
[1] Department of Mathematics and Statistics, Faculty of Science and Technology, Thammasat University, Paholyothin Road, Prathum Thani, Thailand.
E-mail:rsittipong@yahoo.com, Fax: (662)564-4489
[2] Department of Mathematics, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok, Thailand.
[3] Department of Mathematics and Statistics, University of Maryland Baltimore County, Maryland, USA.

Abstract: We propose a mathematical model a single continuous layer of nerve cells with lateral inhibition type of connection functions in a neural network which admits traveling wave front solutions. We obtain a characterization of the wave front shapes that depend on the relationship between the firing threshold potential and a measure of synaptic connection strength. We also consider a nontrivial standing wave front solution at zero wave speed.

A_A0002 DEVELOPMENT OF TEST CASE GENERATION TOOL FOR ACCEPTANCE TESTING

Naris Mingmora, Pichayotai Mahatthanapiwat
School of Computer Engineering, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand.
E-mail:nrming@hotmail.com

Abstract: One of the important aspects of software development to make it reliable and conform to user requirement is testing. The last phase of testing is acceptance testing that uses black box technique to test the requirements specified in the software requirement specification. Actually, the design of test cases for the acceptance testing used considerable time to consider. This research designed and developed tool of test case generation for acceptance testing to help testers to test in this phase. Furthermore, a database was created to record the test cases for the reusability. The research also studied the methodology to store the functional requirement, IEEE Std.830-1993 standard, ESA PSS-05-0 standard, NASA-DID-P200 standard and DOD-STD-498standard. The functional requirement from the software requirement specification will be used to generate test cases for the acceptance testing. The result that test cases can cover the requirement of the end user.

A_A0003 COMPRESSION PROGRAM FOR TRUE COLOR IMAGES

Yaowarat Sirisathitkul, Siriprapa Intaphan and Somrudee Kaewkaw
School of Informatics, Walailak University, 222, Thasala District, Nakhon Si Thammarat, 80160, Thailand.
E-mail: syaowara@wu.ac.th, Fax: (667) 567-2205

Abstract: This program was developed for compression and decompression of true color images. True color images typically use 3 bytes/pixel, each byte for red, green and blue components. Hence, 512x512 pixels true color images have 768 KB file size. As a result, these images require a huge capacity to store and a long time to transfer file over the Internet. The developed program consists of 2 major steps. The first step is divided into 2 phases: a colormap design (256 colors) and an image compression. The second step is a compressed image saving as a .ccg, a new graphic file format proposed in this research. In the colormap design phase, a Median-cut algorithm, one of the most popular algorithm in color image quantization, was developed. The output of this phase is an indexed image and a colormap. After that, the indexed image is sent to a Run Length Encoding (RLE). The RLE is a lossless data compression algorithm that allows the exact original data to be reconstructed from the compressed data. The compressed image is then saved as .ccg file format. The experimental results reveal that the compressed image file size is reduced to one-third of the original size. Moreover, distortion measurements by Mean Square Error (MSE) and Peak Signal-to-Noise Ratio (PSNR) is used to indicate the effectiveness of this program. In most tests, the PSNR value is about 30 dB, meaning that the quantized images are closely resembled the original.

A_A0004 Enhancing aspect-oriented for UML

Siwadol Sateanpattanakul, Pichayothai Mahatthanapiwat
School of Computer Engineering, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand,
E-mail address: sidol.sat@gmail.com, pmh@sut.ac.th

Abstract: Object-oriented paradigm is one of the most widely used techniques for software development at present. Unified Modeling Language (UML) is a standard used for object-oriented software development. However, there are still some limitations in object-oriented software development. The aspect-oriented paradigm will solve these limitations. This research will extend the capability of the UML by including the characteristic of the aspect paradigm. Moreover, XML will be used as a document to transfer data between UML and source code.

A_A0005 HIERARCHICAL FINITE ELEMENT METHOD FOR NONLINEAR HEAT CONDUCTION IN PLATES.

Puntip Toghaw[1], Yongwimon Lenbury, and Hideaki Kaneko[2]
[1] Department of Mathematics, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok, Thailand.
E-mail:tiptoghaw@yahoo.com, Fax: (662)942-8380
[2] Department of Mathematics and Statistics, Old Dominion University, Norfolk, VA., USA.

Abstract: In this paper, we consider a hierarchical finite element method for nonlinear heat conduction problems over three-dimensional plates. Problems considered are nonlinear because the heat conductivity parameter depends upon the

temperature itself. This paper explores a new technique, which transforms a nonlinear parabolic problem to a linear problem. Numerical examples are provided to demonstrate the efficiency of the current technique.

A_A0006 Numerical Methods for the Advection-Diffusion Equation

Montri Thongmoon[1], Robert Mckibbin[2]
[1] Department of Mathematics, Faculty of Science, King Mongkut's University of Technology Thonburi, Pracha-Utid Road, Tungkru, Bangkok, Thailand.
E-mail: mthongmoon@yahoo.com, s6500307@st.kmutt.ac.th
[2] Institute of Information and Mathematical Sciences, Massey University, Albany, Auckland, New Zealand.
E-mail: R.Mckibbin@massey.ac.nz

Abstract: This paper describe numerical models for solving the advection-diffusion equation of the pollutant. The one-dimensional advection-diffusion equation is solved by using the cubic splines interpolation for advection component and diffusion component and solve the same problem by using the finite difference schemes e.g. FTCS method, Crank-Nicolson method. The numerical examples are shown and numerical solutions are compared with the analytical solution. The finite difference FTCS method and the Crank-Nicolson method give better point-wise solutions than cubic spline method.

A_A0007 Using Case-Based Reasoning to Adaptively Select Learning Objects Appropriate for Students

Ruangsak Trakunphutthirak[1], Assoc.Prof.Dr. Pattarachai Lalitrojwong[2]
[1] Department of Mathematics, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.
E-mail: ruangsak@swu.ac.th
[2] Faculty of Information Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.
E-mail: pattarachai@it.kmitl.ac.th

Abstract: In the generation of information and communication technology, thousands of Web-based courses and other educational applications have been made available on the World Wide Web. However, most of them are nothing more than a collection of static hypertext pages. Case-Based Reasoning is based on the idea that one can make use of past experiences in solving similar problems. The features of each case in case bases will extract from the narrative model, the student model and the learning object model. Consequently, this can build the adaptive system that selects personalize learning objects according to the learner's performance.

A_A0008 A study of a differential equation system model of competing bacteria populations in the gastrointestinal tract subject to an inhibitor or an antibiotic

Tippawan Puttasontiphot[a], Yongwimon Lenbury[a], Chontita Rattanakul[b], Sahattaya Rattanamongkonkul[b], John R. Hotchkiss[c] and Philip S. Crooke[d]
[a] Department of Mathematics, Faculty of Science, Mahidol University,Bangkok, 10400, Thailand
[b] Department of Mathematics, Faculty of Science, Burapha University,Chonburi, 20131, Thailand
[c] Department of Critical Care Medicine, University of Pittsburgh,Pittsburgh, PA 15261, USA
[d] Biomathematics Study Group, Department of Mathematics,Vanderbilt University, Nashville, TN 37240, USA

Abstract: We study the effect of an antibiotic or an inhibitor on a combination of sensitive bacteria population and a non-sensitive bacteria population. In this paper, we construct a model that simulates the bacteria-antibiotic dynamics in a gastrointestinal tract. We derive the conditions under which two types of bacteria populations can persist subject to an inhibitor or an antibiotic and, derive the conditions encode the level of minimum inhibitory concentration (MIC) and the minimum antibiotic concentration (MAC) which are important parameters commonly used to quantify the activity of antibiotics against a certain bacterium.

A_A0009 Adaptable learning assistant for item bank management

Atorn Nuntiyagul, Kanlaya Naruedomkul[2] , Nick Cercone[3], Damras Wongsawang[4]
[1] Institute for Innovation and Development of Learning Process, Mahidol University, Thailand
[2] Department of Mathematics, Faculty of Science, Mahidol University, Thailand
[3] Faculty of Computer Science, Dalhousie University, Canada
[4] Department of Computer Science, Faculty of Science, Mahidol University, Thailand

Abstract: We present PKIP, an adaptable learning assistant tool for managing question items in item banks. PKIP is not only able to automatically assist educational users to categorize the question items into predefined categories by their contents but also to correctly retrieve the items by specifying the category and/or the difficulty level. PKIP adapts the "categorization learning model" to improve the system's categorization performance using the incoming question items.

PKIP tool has an advantage over the traditional document categorization methods in that it can correctly categorize the question item which lacks keywords since it adopts the feature selection technique and support vector machine approach to item bank text categorization.

In our initial experimentation, PKIP was designed and implemented to manage the Thai high primary mathematics question items. PKIP was tested and evaluated in terms of both system accuracy and user satisfaction. The evaluation result shows that the system accuracy is acceptable and PKIP satisfies the need of the users.

A_A0010 Developing an Effective Lossless Image Compression by Using Combination of Burrow-Wheeler Transform and Arithmetic Coding Algorithms

A

Nivet Chirawichitchai

* Department of Computer Science, Suan Sunandha Rajabhat University

**Department of Information Technology, King Mongkut's Institute of Technology North Bangkok, Thailand

E-mail address: nivet99@yahoo.com, para@kmitnb.ac.th

Abstract: Lossless compression techniques play an important role in multimedia coding. Especially, Arithmetic Coding is one of the successful techniques for data compression. However, its compression ratio is not high enough for some applications. Therefore, in this research, we have proposed the combination of Burrow-Wheeler Transform (BWT) and Arithmetic Coding to improve the efficiency of compression. In this method, the BWT ,Move-To- Front and Run-length algorithms are used for rearranging the data into a suitable form. Then, the Arithmetic Coding algorithm is applied to these data for coding. In this way, the experimental results show that the compression ratio of the proposed method is higher than that of the traditional coding methods up to 30%.

B1_B0001 Culture Preservation of Hed Krang (Schizophyllum commune) and Hed Khon Noy (Coprinus sp.)
Ratapol Somprasert, Supitcha Chaiwongfun, Vachiraporn Pikulthong and Anong Hambananda
Biology Program, Faculty of Science, Rajabhat Chandrakasem University, Bangkok, Thailand

Abstract: Pure culture from Hed Krang found that mycelium has average diameter of colony 90.0 mm. at 8 days. Growth and development of mycelium found that mycelium has white color. The color of PDA changed to yellow at 7 days. The density of mycelial is highest at 8 days. Pure culture from Hed Khon Noy found that mycelium has average diameter of colony 90.0 mm. at 10 days. Growth and development of mycelium found that the mycelium changed to brown at 10 days and produced brown exudate at 11 days. The color of PDA changed to brown at 10 days. The density of mycelium is highest at 10 days. Culture preservation of Hed Krang in distilled water, mineral oil and PDA found that the mycelial survived 100 % after storage in 1-6 months and storage at -20°C in 1-5 months survived 100 % but decresed 80 % when storage in 6 months. The culture storage methodology average in 1-6 months found that distilled water is the best, mineral oil, PDA and -20 °C have average diameter of colony 60.38, 54.25, 52.43, and 46.71 mm. respectively. Culture preservation of Hed Khon Noy in distilled water and PDA found that the mycelium survived 100% after storage in 1-6 months but decresed 70% when storage in 6 months. In storage at -20 °C there is no growth of mycelium in 1-6 months. The culture storage methodology average in 1-6 months found that in PDA is the best, distilled water and mineral oil have average diameter of colony 52.50, 43.25, and 41.16 mm. respectively.

B1

B1_B0004 The potential cotreatment effect of some plant extracts and some antibiotics on multidrug resistant bacteria
Department of Biology, Burapha University, Bangsaen, Chonburi, 20131
Wisatre Kongcharoensuntorn*, Chanida Sangaewtrup, Wiparat Bootsayatrus, Pantaree Katawee

Abstract: This present study investigated cotreatment effect of three Thai medicinal plants and antibiotics. Overall, the treatment by combining medicinal plant extracts plus antibiotics could inhibit the growth of multidrug resistant bacteria: *Acinetobacter baumannii*, Methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* better than by using plant extracts alone. The cotreatment of three antibiotics with *Dracaena loureiri Gagnep.*, *Mansonia gagei* Drumm., and Myristicaceae fragrans Houtt. exhibited inhibitory effect to multidrug resistant strain of A. *baumannii and and P. aeruginosa* but did not inhibit the growth of MRSA. Those MIC values of dial treatments were lowered than MIC of single treatments 16-32 times, and 4-16 times, respectively. In contrast, there is an antagonistic effect on the use of herbs and antibiotics against MRSA. Also, these results suggested that the combination of using herbs with antibiotics demonstrated the most effective of antimicrobial activity when using low concentration of antibiotics (less than 5 mg/ml).

B1_B0005 A SURVEY OF DICOTYLEDONOUS (POLYPETALAE) OF MEDICINAL WEEDS IN CHONBURI, THAILAND
Pragrom Prayoonrat
Department of Biology, Faculty of Science, Burapha University, Bangsaen, Chonburi 20131, Thailand.
E-mail address: pragrom@buu.ac.th

Abstract: A survey was conducted to categorize medicinal weeds of dicotyledonous (polypetalae) found in Chonburi province, Thailand. Places sampled on 12 areas in 3 districts were residential areas, agricultural lands, plantations, uninhabited areas, along the sea shore, some aquatic habitats and mangrove forest were also included. The result of this survey on dicotyledonous (polypetalae) had 21 families, 53 genera, and 70 species. The medicinal characteristics of each weed species were studied in collaboration with traditional herbal medicine providers as well as from historical manuscripts. Frequency of occurrence and medicinal characteristics of the weeds are discussed.

B1_B011 A Survey of Helminths of Marble Goby, *Oxyeleotris marmorata* (Bleeker, 1852) from Mae-Ngad Somboonchon Reservoir, Chiang Mai Province
Janejira Pubua, Monruedee Chaiyapo, Suksan Chuboon, Chalobol Wongsawad
Department of Biology, Faculty of science, Chiang Mai University, Chiang Mai, Thailand 50200

Abstract: The Helminthological study of 40 Marble Gobies (*Oxyeleotris marmorata* (Bleeker, 1852)) was investigated from Mae-Ngad Somboonchon Reservoir, Mae-Tang district, Chiang Mai province during March to April 2005. Gills, scales, fins, muscle and gastrointestinal tract of fishes were separated and examined for helminthic infection. Three species of helminths were recovered in different parts of the body which indicated as followings; Monogenea, which was found in gills and identified as *Dactylogyrus* sp. with prevalence and intensity were 80% and 12.2 respectively. Another 2 species were acanthocephalan which was found in fish intestine and identified as follows; *Pallisentis* sp. (5%, 0.05) and *Polymorphus* sp. (5%, 0.075).

B1_B0013 Helminthic Infection of Fishes Family Channidae from Bung Boraphet, Nakorn Sawan Province.
Monruedee Chaiyapo[1], Chalobol Wongsawad[1], Pheravut Wongsawad[1], Thanu Marayong[1]
[1] Department of Biology, Faculty of Science, Chiang Mai University 50202
E-mail : chaiyapo@chiangmai.ac.th

Abstract: Helminthic examination of thirty-five channid fishes (*Channa lucius*, *C. micropeltes* and *C. striata*) were collected from Bung Boraphet, Nakorn Sawan province in April 2006. Seven species of helminthes were recovered, as follows: 3 species of cestodes *Senga* sp., *Senga chiangmaiensis* and cystacanths in Order Proteocephala, 2 species of trematodes

Acanthostomum sp. and *Clinostomum* sp., a species of Acanthocephala *Pallisentis nagpurensis* and a species of nematode. The prevalence of helminths in each species were 100, 20, 76, 20, 20, 100 and 20, respectively. The highest intensity of cystacanth in Order Protocephala of *C. striata* was 75.2.

B1_B0015 Learning ability and Color Remembering of Great Hornbill in Captivity at Chiang Mai Zoo

Patchareeyaporn Pan-ya-arj and Narit Sitasuwan
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail: pnanzie@hotmail.com

Abstract: The learning and color-remembering abilities of 3 couples of Great Hornbills (*Buceros bicornis*), in Chiang Mai Zoo, were studied from June 2005 to May 2006. The study used 2 models: color-remembering model and mechanical food box. The first set of model consisted of 4 plastic bowls, which were yellow, red, green, and blue in color. The bowls were covered with paper sheets having the same colors as the bowls, but only the red bowl contained some food. The second model was the mechanical food box, with a lid, containing food. The result of the model testing shown that the Great Hornbills had a good learning ability, and could recognize colors.

B1_B0017 Chemical Analysis of *Aeng* Tae, a Local Food of Tai-Lue in Chiang Rai, Thailand

Jantrararuk Tovaranonte, Wilaiwan Sakdee, Thitikone Kuntama and Ekachai Chukeatirote
School of Science, Mae Fah Luang University, Chiang Rai 57100 Thailand

Abstract: This study was performed to investigate chemical profiles of *Aeng Tae*, a local plant and food of Tai-Lue community. Our results showed that chemical compositions of *Aeng Tae* fresh leaves were as follows: 73.86% moisture, 2.70% fibre, 2.27% protein, 2.11% ash, 21.45% carbohydrate and 0.32% fat whereas those of gelatinous extracts were 98.80% moisture, 0.46% fibre, 0.14% protein, 0.03% ash and 1.02% carbohydrate. Both *Aeng Tae* fresh leaves and gelatinous extracts contained similar amount of reducing sugars (2.72 mg/ml). In addition, the gelatinous extracts were unstable when treating with high temperature (>60°C).

B1_B0019 TAXONOMIC STUDY OF THE FAMILY POLYGONACEAE IN THAILAND

Chortip Kantachot[1], Pranom Chantaranothai[1], and David A. Simpson[2]
[1] Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
[2] Royal Botanic Gardens, Kew, Richmon, Surrey TW9 3AB, UK.

Abstract: The taxonomy of the family Polygonaceae in Thailand is revised. Seven genera, 31 species and 37 taxa are recognised. Keys to genera and species, descriptions and photographs of all species are provided. Two species, *Polygonum pubescens* Blume and *P. viscosum* Buch.-Ham. ex D.Don are new to Thailand.

B1_B0021 PLANKTONIC DIVERSITY IN THE RESERVOIR OF WATERSHED MANAGEMENT DIVISION, CONSERVATIVE AREA ADMINISTRATION OFFICE (SUBREGIONAL OFFICE 16), NATIONAL PARK WILDLIFE AND PLANT DIVERSITY DEPARTMENT, CHIANG MAI PROVINCE

Shamaporn Niwasabutra and Tanu Marayong
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 50200.

Abstract: Diversity of plankton in the reservoir of Watershed Management Division, Conservative Area Administration Office (subregional office 16), National Park Wildlife and Plant diversity Department, Chiang Mai province was investigated during April – May 2006. It was found that total plankton found in this study were 26 species and classified into 3 Phylum described as follows ; Phylum Protozoa, Phylum Rotiferra and Phylum Arthropoda. Total protozoan found were 19 species and classified into 3 Class indicated as followings ; 1) Class Mastigophora 11 species which *Euglena* sp. and *Phacus* sp. represented as dominant species 2) Class Ciliata 5 species which Coleps sp. and *Vorticella* sp showed high population than the others and 3) Class Sarcodina 3 species, most of them were *Arcella* sp. but no any sarcodinid showed as dominant species. According to Phylum Rotifera, total rotifers found were 4 species and *Rotaria* sp. (Class Digononta) represented as dominant species Whereas Phylum Arthropoda, total arthropods found were only 3 species and species represented as dominant species was belonged to *Cyclops* sp (Class Crustacea). From over all, planktonic composition based on number of species was indicated as followings; Phylum Protozoa (73.08%), Phylum Rotiferra (15.38%) and Phylum Arthropoda (11.54%) respectively.

B1_B0022 DIALECTS OF WHITE-CRESTED LAUGHING THRUSH (Garrulax leucolophus)

Suwimol Chinkangsadarn[1], Narit Sitasuwan
[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract: White-crested Laughing Thrush's dialect analysis was done at 3 main sites in districts of Maung, Doi Sa-ked and Chiang Dao, Chiang Mai province from August 2005 to March 2006. The vocal communication was recorded, transfered into sonagram, and analyzed for song patterns. Behaviours of birds were observed and recorded. The analyses were performed and comparisons were made among the birds within and between the sites. It was found that there was not true song in White-crested Laughing Thrush, but only subsongs and calls. According to the sonagram study, the birds sang with a constant frequency ranging between 1 to 10 kHz. It was found that 10 types and 57 sub types of repertoire divided by

structure of elements were found. Two types of subsongs were found for contact and mobbing, and four types of calls for alert, alarm, excitement and begging.

B1_B0027 The Toxicity of Leave Crude Extracts from Neem Tree (*Azauirachta indica* Juss.) and Barlic (*Allium sativom* L.) on Mortality rate of Glogen Apple Snail (*Pomocea* sp.)

Rachadaporn Benchawattananon and Umpriwan Boonkong
[1] อาจารย์ Applied Biology Programe, Faculty of Science and technology, Loei Rajabhat University, Loei, Thailand
Email: rachadaporn2000@yahoo.com
[2] นักศึกษา General science Programe, Faculty of Science and technology, Loei Rajabhat University, Loei, Thailand

Abstract: The Toxicity of Leave Crude Extracts from Neem Tree(Azauirachta indica Juss.) and Barlic(Allium sativom L.) on Mortality rate of Golden Apple Snail(Pomocea sp.) were studied. At concentration 50, 250, 500, 750 and 1,000 milligrams per liter were tested on Golden Apple Snail for 3 replications.High concentration of Neem Tree 1,000 milligrams per liter killed Golden Apple Snail 95.83% in 96 hours and high concentration of Barlic 1,000 milligrams per liter killed Golden Apple Snail 91.66% in 96 hours as compare to distill water control. Therefore, Neem Tree and Barlic are applicable for biological control of Golden Apple Snails.

B1_B0029 CHARACTERISTICS OF *SAPROLEGNIA DICLINA* ISOLATED FROM EGGS OF TILAPIA (*OREOCHROMIS NILOTICUS* LINN.)

Kwanprasert Panchai[1], Chutima Hanjavanit[1] and Nilubon Kitancharoen[2]
[1] Applied Taxonomic Research Center (ATRC), Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
[2] Department of Fishery, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
Corresponding e-mail: chuhan@kku.ac.th

Abstract: Tilapia eggs (*Oreochromis niloticus* Linn) with fungal hyphae were collected from Inland Fisheries Research and Development Center, Muang District, Khon Kaen province during June-July 2005. Fungal species have been isolated and identified. Biological characteristics such as effects of temperature, sodium chloride (NaCl) and pH on mycelial growth were studied. From the morphological study, the fungus was identified as *Saprolegnia diclina*. Biological characteristic examination of the fungal species showed that the optimum temperature for mycelial growth was 25°C and it was able tolerate up to 30 ppt NaCl. In addition, the vegetative growth of S. *diclina* could grow well at pH range of 7.0-9.0.

B1_B0034 ROLE OF 20-HYDROXYECDYSONE HORMONE ON THE DEVELOPMENT OF WING IMAGINAL DISKS IN *OMPHISA FUSCIDENTALIS* IN VITRO.

Kitti Tanmuangpak[1] and Tippawan Singtripop[2]
[1] Program of Biology, Faculty of Science and Technology, Loei Rajabhat University, Loei, Thailand.
E-mail:kittizipzip@hotmail.com, Fax: 0-4283-5238
[2] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Abstract: The final instar larvae of the bamboo borer (*Omphisa fuscidentalis* : Pyralidae ; Lepidoptera) are in a period of developmental arrest called larval diapause. The previous studies showed that topically applied 20-hydroxyecdysone (20E) induced pupation of the diapause larvae. Accordingly, we determined the effect of application 20E on the size of the wing imaginal disks and protein amounts in individual disks (in vitro). Culture of the disks in Grace's medium containing various amounts of 20E (0.05, 0.1, 0.25 and 0.5 µg/1 ml) revealed that 20E induced morphological changed of the disks. In conclusion, 20E is capable of inducing the development of disks both in vivo and in vitro.

B1_B0041 HORMONAL EFFECTS ON CELL CYCLE IN CULTURED WING IMAGINAL DISC OF SILKWORM, Bombyx mori.

Pimonphan Sereewattanachai[1], Tippawan Singtripop[1], Sho Sakurai[2]
[1] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[2] *Division of Developmental Biology, Graduate School of Science and Technology, Kanazawa University, Kakumamachi, Kanazawa 920-1192, Japan*

Abstract: This study was undertaken to find the factors which effect on wing imaginal disc differentiation in lepidopteran wing discs in vitro. Wing discs of silkworm were cultured in medium containing an optimal concentration of 20-hydroxyecdysone (20E) and/or bombyxin, FBS (fetal bovine serum) including the oxygen supply. After 24 h in culture, they were observed as S and M phase cell by immunocytochemistry and their numbers were counted by NIH image. The results showed that the number of S and M phase cells increased in the 20E alone and 20E with Bombyxin on the late fourth instar larvae and the highest increase on the early fifth instar larvae. FBS and oxygen supply also served to maintain the imaginal disc differentiation. These results suggest that the the number of S and M phase cells depend on the developmental stage and optimal concentration of 20E and bombyxin including growth factors can induce cell proliferation followed by imaginal differentiation.

B1_B0042 IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) OF GENES IN HEMOCYTE, OVARIES AND TESTES OF THE GIANT TIGER SHRIMP *Penaeus monodon*

Sirikant Prasertlux[1], Sirawut Klinbunga[2,3] and Piamsak Menasveta[2,4]

[1] Programarine of Biotechnology,[2] Center of Excellence for Marine Biotechnology,[4] Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[3] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani 12120

Abstract: Single nucleotide polymorphism (SNP) in functionally important genes of the giant tiger shrimp (*Penaeus monodon*) was examined by SSCP. A total of 108 pairs of primers were designed from ESTs of hemocyte, ovary and testis libraries and 49 primer pairs generated positive amplification product for which 29 gene segments contained introns. SSCP patterns of 43 genes were examined using genomic DNA of wild *P. monodon* (N = 15) and 37 of which exhibited polymorphic SSCP patterns. Nucleotide sequences of an individual representing each SSCP genotypes of *x-box protein, NADP-dependent leukotriene-12-hydroxydehydrogenase (ADRAB-F), Phosphatidylinositol 4 kinase, RuvB-like protein 2, phosphatidylserine receptor, Adenine nucleotide translocator 2)* were examined and showed compatible results with those from SSCP analysis. Subsequently, 19genes were preliminary screened against 10 individuals of a full-sib family of *P. monodon* and revealed polymorphic SSCP patterns. Association between SNP by SSCP and the growth rate of a *P. monodon* family (*N* = 75) is being determined. RT-PCR of *ADRAB-F* which is also recognized as *15-oxoprostaglandin 13-reductase* showed differential expression patterns in ovaries of broodstock-sized *P. monodon* indicating that this gene may play an important role in reproductive maturation and ovarian development of female *P. monodon*.

B1_B0043 CRYOPRESERVATION OF *DENDROBIUM GRATIOSISSIMUM* RCHB.F PROTOCORMS BY ENCAPSULATION-DEHYDRATION METHOD

Praweena Maneerattanarungroj[1], Sumontip Bunnag[2] and Montira Montatong[2]

[1] Doctoral student, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
E-mail: p_raweena@yahoo.com

[2] Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: *Dendrobium gratiosissimum* Rchb.f. is wild orchid of Thailand which is rare species. Cryopreservation is an alternative and important tool for *ex situ* preservation of orchid germplasm. In this study, protocorms of *D. gratiosissimum* were induced and cultured on solidified New Dogashima (ND) medium containing 0.3 mg/l NAA for 2 months. These protocorms were used as explants in cryopreservation by encapsulation – dehydration method. The highest percentage regrowth of cryopreserved explants was observed when precultured in liquid ND medium with 1 mg/l ABA for 1 week and then dehydrated with silica gel in laminar air-flow for 5 or 6 h prior to immersion in liquid nitrogen for 1 h. Viability tests were investigated by TTC assay and survival rate after recovery for 2 weeks. The TTC assay percentage was about 50% and the survival rate was 40%. However, effective protocol for increasing the recovery ability of cryopreserved protocorms of *D. gratiosissimum* are needed.

B1_B0048 ISOLATION AND CHARACTERIZATION OF FULL LENGTH cDNAS AND GENES OF *CALPONIN 1, DAD1* AND *THIOREDOXIN PEROXIDASE* OF THE GIANT TIGER SHRIMP *(PENAUS MONODON)*

Arun Buaklin[1], Sirawut Klinbunga[2,3] and Piamsak Menasveta[3,4]

[1] Programa of Biotechnology, [2]Center of Excellence for Marine Biotechnology, [4]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[3] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani 12120
e-mail: sirawut@biotec.or.th

Abstract: The full length cDNA of homologues of *calponin 1* (ORF = 561 bp encoding a polypeptide of 187 amino acids) *defender against cell death 1* (DAD1; ORF = 345 bp, 115 aa), and *thioredoxin peroxidase* (ORF = 591 bp, 197 aa) were isolated thorough an EST approach. Gene specific primers were designed and used for identification of their full length genes by genome walking analysis. *Calponin 1* contained of 3 exons (185, 206 and 170 bp) and 2 introns (214 and 306 bp). DAD1 contained 2 exons (214 and 131 bp) and 1 intron (215 bp). *Thioredoxin peroxidase* contained 3 exons (258, 162 and 171 bp) and 2 introns (89 and 111 bp). The expression levels of *thioredoxin peroxidase* and *DAD1* in ovaries were greater than those in testes of juvenile *P. monodon* ($P < 0.05$). In contrast, *calponin 1* was more abundantly expressed in testes than ovaries ($P < 0.05$). The time-course expression levels of these genes in ovaries and hemocytes of juvenile *P. monodon* females after temperature stress (33 °C for 6 hours) were semiquatitatively examined. Only *thioredoxin peroxidase* in ovaries of stressed shrimps displayed significant up-regulation at 6 hours post temperature stimulus ($P < 0.05$).

B1_B0050 Taxonomic Study on the Genus Piper L. in Thailand

Chalermpol Suwanphakdee[1], Pranom Chanataranothai[2] and Sumon Masuthon[3]

[1] King Monkut's Institute of Technology Ladkrabang, Chumphon campus, Prathiu, Chumphon province 86160

[2] Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

[3] Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900

Abstract: The genus *Piper* L. in Thailand is taxonomically revised. Thirty-six species, including two varieties, are recognized. Among these, four species are introduced, eight species await further study as there are inadequate specimens for description of new taxa. Twelve taxa are recorded for the first time in Thailand. Key to species with full descriptions, distributions and uses as well as illustrations and color photographs are provided.

B1_B0051 Effects of low amount of Haematocrits on some Physiological aspect of Plaice (*Pleuronectes platessa*)

Itsara Intanai
Department of Sciences, Faculty of Sciences and Technology, Prince of Songkla University, Pattani 94000, Thailand.

Abstract: A study was made of the effects of low haematocrits on ventilation, blood ventillation, acid-base status and heart rate in the plaice (*Pleuronectes platessa*). Unrestrained, quiescent plaice, maintained normal level of O_2 uptake in a 15 l experimental at 13-14 °C. At haematocrits reduced from 16% to 8 %, the heart rate was increased from 29.25 ± 2.74 to 33.96 ± 1.57 no. min-1. The blood pH was increased from 7.58 ± 0.01 to 7.70 ± 0.01. The low oxygen concentration did not affect gill ventilation volume, ventilation rate and total CO_2 in the blood.

B1_B0055 Nest Dispersion and Genetic Structure of the Dwarf Honey Bee, Apis florea

Wandee Wattanachaiyingcharoen[a], Benjamin P. Oldroyd[b], Siriwat Wongsiri[c]
[a] *Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000*
[b] *School of Biological Sciences,A12, University of Sydney, New South Wales, Australia,2006*
[c] Faculty of Technology, Mahasarakham University, Mahasarakham and Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330

Abstract: The dwarf honey bee, *Apis florea*, is distributed throughout Thailand and is present in both natural and disturbed areas. The density of nests is dependent on the availability of floral resources and suitable nesting sites. *A. florea* nests tend to have an aggregated spatial distribution. We located and mapped the precise location of 3 aggregations of *A. florea* in urban areas in Phitsanulok province. Adult bees were collected and investigated using microsatellite markers to determine the relatedness of colonies within and between the aggregations. The results revealed that within aggregations, colonies are not closely related.

Why should unrelated colonies aggregate for nesting? Aggregations are unexpected because of increased risk of predation and disease transmission. We speculate that colonies benefits from aggregation because of the increased availability of unrelated males for mating. This hypothesis is supported by our finding of low relatedness of nests within aggregations.

B1_B0057 DIVERSITY OF EDIBLE AQUATIC PLANTS IN THE WETLAND OF NONG-HARN KUMPAWAPEE, UDONTHANI.

Seewapong Chamratpan
Faculty of Science, Udonthani Rajabhat University, 64 Taharn Road, Udonthani, 41000.
E-mail: s_chamratpan@yahoo.com, Fax: (664)2341-615

Abstract: The survey of edible aquatic plants in the wetland of Nong-Harn Kumpawapee, Udonthani, Thailand was taken through the 3 seasons of the whole year. The study areas were Bandiam, Non-Tan, Ban Don-Ngeon and Don-Keaw. Thirty four species of edible aquatic plants were found in Nong-Harn. Two species were algae, five species were ferns and 28 species were angiosperms. Plant parts used for eating were young petioles, peduncles, flowers and shoots. Most of them were eaten fresh and some were boiled before they were eaten. Some floated such as *Nymphiodes indicum*. Some suspended such as Chara, Nitella, *Utricularia aurea* and hornwort. Some submerged such as lotus and water lily. The most common in Nong-Harn were lotus and water lily. Some grew in shallowness such as *Colocasia esculenta, Limnocharis flava, Eichhornia crassipes, Ludwigia adscendens, Monochoria elata*, and *Sagittaria sagitifolia*.

B1_B063 PRODUCTION OF MONOCLONAL ANTIBODIES SPECIFIC TO VITELLOGENIN AND ZONA RADIATA PROTEINS FOR THE DETERMINATION OF XENOESTROGEN IN WATER

Pajongsuk Sutarut[1], Narongsak Puangiarp[2,*], Siwaporn Longyant[4], Paisarn Sithigorngul[4], and Piamsak Menasveta[2]
[1] Program of Biotechnology, [2]Center of Excellence for Marine Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok, 10330 Thailand;
[3] National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA, 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani 12120;
[4] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok,10110 Thailand;

Abstract: Monoclonal antibodies specific to vitellogenin (VTG) and zona radiata protein (ZRP) of Greenback Mullet (*Liza subviridis*) were generated using proteins extracted from unfertilized eggs as immunogens. Hybridomas producing antibodies that bound to VTG and ZRP in both native and denatured forms were selected by dot-blotting and were characterized for their specificities by Western blot analysis and Immunohistochemical staining. Isotype and subisotype of antibodies were identified by sandwich ELISA. It was found from the result that 11 monoclonal antibodies specific to VTG can be grouped into 6 categories according to their binding capabilities to different stages of antigens. Most of monoclonal antibodies bound to both native and denatured antigens while one group of them (VTG-313, 530) bound to only native antigens. For oocyte immunohistochemical staining, 2 groups of monoclonal antibodies were able to cross react to all stages of vitellogenic cells.

All monoclonal antibodies belong to the IgG_1 isotype except one that belongs to the IgG_2 isotype. For the production of monoclonal antibodies specific to ZRP, 11 antibodies were specific to both native and denatured forms of ZRP in blood serum of mature female mullets. VTG and ZRP syntheses in juvenile mullets injected with esradiol were determined. The result indicated that the levels of VTG and ZRP were significantly increased in responding to the increase level of E2. This indicates that detection of VTG and ZRP in Greenback Mullet using antibodies is an effective method for determining xenoestrogenic effect in water.

B1_B0082 SPECIES AND INDIVIDUAL RECOGNITION OF ORIENTAL-MGPIE ROBIN *(Copsychus saularis)* BY SONG VARIATION.

Aniroot Dunmak, Narit Sritasuwan
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
E-mail:Dunmak999@yahoo.com.

Abstract: Thirty different song types were recorded and analyze from population of Oriental-Magpie Robin (*Copsychus saularis*). The study was carried out at Lamphoon province from April 2000 to March 2001. Song was analyzed using spectrogram (AVISOFT©) focusing on difference in song structure and syntax. Species recognition can be identified by 1). Frequency of the song ranging between 2 to 6 kHz 2). The presence of core element within the first second of each strophe and 3). The repetition use of strophes (>2 strophes for each song). Individual recognition can be observed from character and different numbers of specific element at the terminal end of each strophe.

B1_B0085 HORMONAL CONTROL OF PROGRAMMED CELL DEATH IN THE BAMBOO BORER (*Omphisa fuscidentalis*)

Tippawan Yasanga and Tippawan Singtripop
Department of Biology Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
E-mail:timm_y181@hotmail.com

Abstract: Prothoracic gland, anterior silk gland and salivary gland are a larval specific tissue of insects which are eliminated by programmed cell death (PCD) after pupation. The present studies focus on the effect of juvenile hormone (JHA) and 20-hydroxyecdysone (20E) on the PCD of prothoracic gland, anterior silk gland and salivary gland both in vivo and in vitro culture. The result showed that JHA application stimulated PCD of prothoracic gland and anterior silk gland on day 3 and stimulated PCD of salivary gland on day 9 after hormone application. Injection of 20E stimulated the PCD of prothoracic gland, anterior silk gland and salivary gland on day 1 after hormone application. Morphological and histological changes showed characteristics of apoptosis cell death. Furthermore, 20E induced PCD of prothoracic gland, anterior silk gland and salivary gland after 24 h. of culture. These results indicate that JHA and 20E effect on the PCD of prothoracic gland, anterior silk gland and salivary gland of the bamboo borer both in vivo and in vitro.

B1-B0088 THE SURVEY OF MOSQUITO BREEDING SOURCE IN TYRES AND APPLICATION OF GIANT MOSQUITO LARVAE TO DECREASE *AEDES AEGYPTI* POPULATION

Wichai Srisukha, Paitoon Leksawasdi
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50100, Thailand; telephone: +66 1 881 6055, fax: +66 53 892 259,
e-mail: noppol@hotmail.com

Abstract: The survey of mosquito breeding source in Chiang Mai, Lampoon and Lampang provinces found 6 species and 1 genus of larvae, namely, *Toxorhynchites splendens*, *Aedes albopicta*, *Ae. Aegypti*, *Culex quinquefaciatus*, *Cx.* (Eumelanomyia) *brevipalpis*, *Cx. vishnui* and one genus of Lutzia which was reported only once in Lampoon province during June. A number of giant and house mosquito larvae (*Aedes aegypti*) were spotted during rainy season, except those in genus Lutzia. The prominent characteristics of adult giant mosquito include large size, curved mouth part in the backward direction and concave marking at the wing edge above CuA line. The average periods of development of giant mosquito larvae in each stage and its cocoon were 2 ± 0.47, 3.13 ± 0.73, 4.34 ± 0.99, 3.95 ± 0.90 and 5.16 ± 0.90 days respectively. The average number of preys in the feeding of giant mosquito larvae from the first to fourth stage were 9.57 ± 2.74, 16.47 ± 5.12, 37.84 ± 10.33 and 53.12 ± 7.75 respectively.

B1_B0091 COMPARATIVE MICROMORPHOLOGY OF ACHENES OF THE GENUS ELEOCHARIS R. Br. AND MAPANIA Aubl. (CYPERACEAE) IN THAILAND

Supawadee Pechcoth, Achra Thammathaworn
Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 4002, Thailand.
E-mail address: pechcoth@yahoo.com, achara@kku.ac.th

Abstract: Micromorphology of achenes inner wall of nine taxa of *Eleocharis* and five taxa of *Mapania* in Thailand was studied and compared using scaning electron microscopy to identify good taxonomic characteristics. The studied taxa were classified into 3 groups based on epidermis of achene inner wall; (1) reticulate surfaced with lumen: *E. dulcis* var. *dulcis*, *E. macrorrhiza*, *E. retroflexa* ssp. *chaetarina* and *E. spiralis* (2) reticulate surfaced without lumen: *E. acutangula*, *E. congesta* var. *japonica*, *E. geniculata*, *E. ochrostachys* and *E. tetraquetra* and (3) sculptured surface: *M. cuspidata*, *M. enodis*, *M. kurzii*, *M. palustris* and *M. tenuiscapa*. Furthermore, the configuration of the anticlinal cell wall, the presence or absence of lumen pit, lumen depressions and the contour of the cell lumen provide the good taxonomic characters for *Eleocharis* taxon identification.

B1_B0092 Recovery of Lichen Diversity during Forest Restoration in Northern Thailand

Aurathai Phongchiewboon, Wanaruk Saipunkaew, Stephen Elliott and Sutthathorn Suwannaratana
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
E-mail: aomaura@gmail.com

Abstract: Recovery of lichen diversity during forest restoration was studied in Northern Thailand. Four tree species were randomly selected. There were 120 sampling trees from three reforestation plots planted in Ban Mae Sa Mai Village, Amphur Mae Rim, Chiang Mai Province by Forest Restoration Research Unit (FORRU), and 30 trees from nearby natural forest. The total number of 795 epiphytic lichen specimens was collected, only crustose and foliose lichen were found. Six orders, 14 families, 31 genera and 70 species were identified. Lichen diversity was determined by using Shannon's diversity index. Evenness and species richness were also determined. It was found that lichen diversity increased with increasing in reforestation age. Sorensen's index was used for similarity and different indices of species components in each plot. Environment factors such as temperature, light intensity, pH of bark, and elevation above sea level had influence on distribution and diversity of lichen.

B1_B0093 EFFECT OF TEMPERATURE ON PETAL COLORATION OF *PERESKIA BLEO* (KUNTH) DC.

Arkarachai Fungtammasan[1], Kanyaratt Supaibulwatana[2]
[1] Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.
E-mail: u4705270@student.mahidol.ac.th
[2] Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

Abstract: Flower pigment is the secondary metabolite that influenced by cultivar, organ, and stage of plant growth and development. It can be affected by various environmental factors. Study on the physiological expression responded to environmental signals is significantly important for scientists to further monitor the biochemical metabolites at cellular level. In this study, the flowers of *Pereskia bleo* demonstrated the alteration in petal color according to the change in environmental climate was used as a model to investigate the effect of environmental factors on petal coloration. Flowers coloration in natural environment was observed during June 2005 to February 2006, whereas the data of average rain, temperature, and photoperiod were collected throughout the experimental period. It was found that temperature plays its important roles for coloration of *Pereskia bleo*'s petals. During the flower development, the maximum temperature that was continuously lower than 30 °C for a period could affect to induce pink-color petals rather than the orange color that generally present. Irregular development of petal color presenting partial pink at the petal edge was observed according to uncontrollable changes in climate. The development of petal color in flower bud was determined using the growth index of flower bud's size. The branches with flower buds were picking up prior to develop the color in order to validate the effect of temperature using control environmental chamber. The petal color of flower buds treated with 20 °C were completely pink, whereas those incubated under 25 °C were orange. Total anthocyanin content of pink petal was higher than orange petal. This investigation reveals the effect of temperature on biochemical change of flower pigmentation. The types of and regulation of enzymes involve in anthocyanin biosynthesis are needed to be further evaluated.

B1_B0116 EVALULATION OF THE TOXICITY AND EFFICACY OF SODIUM CHLORIDE AND HYDROGEN PEROXIDE FOR ANTIFUNGAL ON EGGS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AND COMMON CARP (*CYPRINUS CARPIO*)

Charuwan Rakamnee[1], Chutima Hanjavanit[2], Nilubol Kitancharoen[3]
[1] Program of Biology, Faculty of Science and Technology, Loei Rajabhat University, Loei, Thailand,
E-mail:charuwan_L@hotmail.com.
[2] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
[3] Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

Abstract: Toxicity of sodium chloride at a concentration range of 0-50 g/L and hydrogen peroxide at a concentration range of 0-1,500 ml/L were individual test on live and not infected of fungal on African catfish and common carp eggs for 60 minutes at 25 °C. It was found that 10-50 g/L of sodium chloride had toxicity to both kinds of egg, whereas concentrations of hydrogen peroxide 250 and 500 ml/L have toxicity to African catfish and common carp eggs, respectively. From *in vitro* test, treatment with 250 ml/L of hydrogen peroxide had no effectiveness on inhibition of hyphal growth and zoospore germination of four fungal isolates from both kinds of fish egg; *S. diclina* MHSK 01, sp. *Achlya sp.* MHSK 06, *A. ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12

B1_B0118 Eriocaulaceae in Eastern Thailand

A. Prajaksood* and J.A.N. Parnell**
* Applied Taxonomic Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
**Department of Botany, Trinity College, Dublin 2, Ireland

Abstract: Species diversity of Eriocaulaceae in Eastern Thailand is a part of Flora of Thailand Project. In Asia, there is only a single genus present, *Eriocaulon* L. Consequently, in the family is presented by this genus. The taxonomic study was undertaken, 18 species, 19 taxa were found.

B1_B0123 THE GENUS TARENNA GAERTN. (RUBIACEAE) IN THAILAND

Wirot Kesonbua, Pranom Chantaranothai and Amornrat Prajaksood
Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

Abstract: A taxonomy study of the genus *Tarenna* Gaertn. in Thailand were carried out between June 2004 and July 2006. Both dried specimens from herbaria and living specimens from field study were examined. There are 23 species and one variety, two of which are new records from Thailand, i.e. *T. adangensis, T. angustifolia, T. cinerea, T. collinsae, T. corymbosa, T. costata, T. fragrans, T. harmandiana* (new record), *T. hirsute, T hispidula, T. hoaensis, T. insularis, T. longifolia, T. membranacea* (new record), *T. pillosa, T. puberula, T. pulchra, T. quocensis, T. quocensis* var. *laotica, T. sakae, T. stellulata, T. valida, T. vanprukii and T. wallichii.* Additionally, *T. cinnamomea, T. elliptica, T. pauciflora, T. pubescens* and *T. viridis are* found to be synonym of *T. pulchra, T. collinsae, T. adangensis, T. cinerea and T. puberula*, respectively.

B1_B0125 DEVELOPMENT OF OVULE, MEGASPORE AND MEGAGAMETOPHYTE OF FICUS HISPIDA (MORACEAE)

Wattana Tanming and Pranom Chantaranothai
Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand.

Abstract: The development of the ovule, megaspore and megagametophyte of *Ficus hispida* L.f. is investigated. The female flowers from different size of inflorescence were fixed in FAA 50%, then washed with tap water and dehydrated with alcohol series. Ovaries were cleared using modified BB-4 ½ solution and observed through a differential interference contrast microscope. The ovule is subapical placentation, anatropus, crassinucellate and bitegmic. The micropyle is formed by both integuments. Megaspore mother cell develops from the parietal cell and undergoes the meiosis, resulting in a linear tetrad which the chalazal one becomes a functioning megaspore. The megagametophyte is monosporic and its development is of the Polygonum type. Polar nuclei fused before pollination in mature gametophyte.

B1_B0126 Species diversity of saline water protozoa from Haui Nam Kem, Khon Kaen Province.

Nawarat muanglen[1] andPinich Wangsomnuk[2]
[1] Graduate of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
E-mail address: Muanglen@yahoo.com
[2] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
E-mail address: Pinwan@kku.ac.th

Abstract: Species diversity of saline water protozoa from Haui Nam Kem, Ban Tum Subdistrict, Muang District, Khon Kaen Province was studies. Nine sampling point were collected in the month of March and April 2006. Using plankton net at the mesh size of 20 µm. Salinity values fluctuated between 0.1 ppt and 1.3 ppt. Protozoa were identified with light and scanning electron microscopy (SEM). Most abundantly distributed genus of protozoa were found to be *Arcella* sp., *Actinobolina radians, Chlamydomonas* sp., *Cristigera phoenix, Euglena acus, Phacus alata, Phacus pleuronectes,* and *Trachelomonas* sp. with the exception of *Difflugia lebes* at less abundantly found. Types of protozoa with high tolerance to water salinity up to 1.3 ppt were *Actiophrys sol, Amoeba* sp., *Centropyxis minuta, Centropyxis* sp. and *Cyclidium glaucoma* These protozoa species could be used as an indicator of saline water quality.

B1_B0128 THE TREND OF LABORATORY ANIMAL USING IN THAILAND.

Thanee Sukglin*[1], Viphanee Chinchamnan[2], Kanchana kengkoom[1]
[1] Production and Service Division,
[2] Administration Division, National Laboratory Animal Centre, Mahidol University, Nakornpathom 73170, Thailand;
e-mail address: thaneesukglin@hotmail.com

Abstract: The objective of this study is to estimate the laboratory animals' production for next five years of National Laboratory Animal Centre (NLAC), Mahidol University; the biggest animal center in Thailand. Three years records of laboratory animals' service from NLAC since year 2003 to 2005 were analyzed. The laboratory animal in this studied include 11 strains from 5 species as ICR outbred mice, BALB/cA, C3H/HeN, C57BL/6J and DBA/2J inbred mice, BALB/c-nu (NUDE) mutant mice, Sprague dawley and Wistar rat, Syrian hamster, Dunkin Hartley guinea pig and New Zealand White Rabbit. The result shown that mostly animal used was increase continuously and trend to gain in demand continuously. Only C3H/HeN and DBA/2J inbred mice were slightly and never used respectively and in future demand of them still very low. The main reasons were specification and suitable of each strain.

B1_B0129 Short Hydrophobic Region Lead the PRSV P3 Protein to Endoplasmic Reticulum

Sarasate Flamtanasate[1], Sunee Kertbundit[2], Sakol Panyim[1, 3], Yun-Kiam Yap[1]
[1] Institute of Molecular Biology and Genetics, Mahidol University
[2] Department of Biochemistry, Faculty of Science, Mahidol University
[3] Department of Plant Science, Faculty of Science, Mahidol University

Abstract: Papaya Ringspot Virus (PRSV) is an ssRNA virus that causes severe disease in papaya and some cucurbits

plants. PRSV genome encodes for 1 structural and 8 non-structural proteins. P3, consisting of 345 amino acid residues, is one of the least characterized non-structural proteins that both functions and localization still remained unclear. In this study, the onion epidermal cells were used to express PRSV P3 fused with green fluorescent protein (GFP) transiently. The results indicated that P3-GFP protein was localized in the endoplasmic reticulum (ER). Fusion of 45 amino acids hydrophobic region located towards the C-terminal of P3 with GFP leaded to target the GFP fusion protein to ER. Based on the results, it proposed that PRSV P3 may play a crucial role in viral replication by targeted replication complex to ER via 45 amino acids hydrophobic region

B1_B0137 SPECIES DIVERSITY OF SOIL TESTATE AMOEBAE (PROTOZOA) IN KHOK PHU TA KA, AMPHOE PHU WIANG, KHON KAEN PROVINCE

Jintana Homkailat [1]and Pinich Wangsomnuk[2]
[1] Graduate student, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail: jhomkailat@hotmail.com
[2] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

Abstract: The survey of testate amoebae was conducted from 11 different sites of Khok Phu Ta Ka, Amphoe Phu Wiang, Khon Kaen province. Soil samples were collected during dry season (January to March 2005) and rainy season (August to October 2005) to analyze for numbers and species of testae with Non-Flood Petri dish Method and Flood Petri dish Method, respectively. The parameters, moisture, pH, organic matter, nitrogen, C:N and soil texture were recorded. A total of 16 genus and 26 species was identified. Eleven species were found in high nutrient soil, whereas *Phryganella paradoxa* was found in poor organic soil. Four species *Arcella discoides*, *Difflugia tuberculata*, *Cyclopyxis eurystoma* and *Trinema linear* were frequently found in different sites. Species diversity of testate amoebae may be used as bioindicator of soil.

B1_B0138 TOXICITY EFFECTS OF RESORCINOL ON THE FRESHWATER OLIGOCHATE, *Tubifex tubifex*

Nalena Praphairaksit[1*], Junyaporn Oomong[1]
[1*] Department of Biology, Faculty of Science, Srinakarinthvirot university, Bangkok 10110. Thailand;
e-mail address : nalena@swu.ac.th

Abstract: Resorcinol is a chemical used in an antiseptic disinfectant an anti-dandruff agent in shampoo in sunscreen cosmetics.and in hair coloring. This chemical is released into the environment without any concern of how it will effect the environment. In this study we use an aqutic worm as a model to study the effects both lethal (LC_{50}) and sublethal levels of resorcinol on vasculotoxicity and behavioral responses exposure. The tests were carried out and compared between before and after 30 min and 1 hours exposure of the chemical. 5 sublethal concentrations of 230, 210, 190, 170, 150 and 130 ppm were selected. The results shows the statistically decrease in blood pulsation rate of the worms in all concentration tested right from the beginning (paired-t test $p < 0.05$). The worms moved less frequently and had convulsion of the muscles. The increase in the strength of the convulsion corresponded with the increase in resorcinol concentration.

B1_B0150 BIODIVERSITY OF THE LICHEN FAMILY CLADONIACEAE AT PHU HIN RONG KLA NATIONAL PARK, THAILAND.

Sittiporn Parnmen, Natsurang Homchantara and Piboon Mongkolsuk
Lichen Research Unit, Department of Biology, Faculty of Science, Ramhkamheang University
E-mail: sparnmen@gmail.com, Tel. (02) 3108410 # 132, Fax: 02 3108416

Abstract: Study on biodiversity of lichens in the family Cladoniaceae was explored at Phu Hin Rong Kla National Park during 2003 - 2005. Collected 326 samples were identified into 2 genera, 13 species and 3 sections. They comprised of *Cladia aggregata* (Swartz) Nyl. and 3 sections of genus *Cladonia*. The section *Cladonia* had 10 species, namely *Cladonia borbonica* Nyl., *C. rappii* A.Evans, *C. ramulosa* (With.)Laun., *C. singhii* Ahti & Dixit, *C. submultiformis* Asah., *C. fruticulosa* Kremp., *Cladonia* cf. *awasthiana* Ahti & Upreti, *C. ochrochlora* Flörke, *C. maurtiana* Ahti & J.C.David and *Cladonia* ph.1. The section Cocciferae had 2 species, including *Cladonia macilenta* Hoffm. and *C. rubricapitata* Ahti, ined. And the section Perviae had one species which was *Cladonia* aff. *crispatula* (Nyl.)Ahti. The *Cladonia rubricapitata* was described as a new record in Thailand. Three species were expected to be new species consisted of *Cladonia* cf. *awasthiana*, *Cladonia* aff. *crispatula* and *Cladonia* ph.1. Species composition varies among different forest types. The highest diversity was found in lower montane shrub forest (28%), lower montane rain forest (28%) and lesser in lower montane oak forest (25%), the lowest diversity was found in the lower montane oak-pine forest (19%) There has been no report on the finding of 3 Cladonia sections in mixed deciduous forest and tropical rain forest. Most of specimens were found on rock and soil, rarely on bark. Most secondary metabolites found were depsides, depsidones, anthraquinones, dibenzofurans and dibenzofuranoid derivatives.

B1_B0155 BIODIVERSITY OF THE LICHEN FAMILY USNEACEAE AT PHU HIN RONGKLA NATIONAL PARK.

Natwida Dangphui, Piboon Mongkolsuk, Natsurang Homchantara, Kajonsak Vongchewarat and Kawinnat Buarueng
Lichen Research Unit, Department of Biology, Faculty of Science, Ramhkamheang University
E-mail: natwida_new@gmail.com, Tel. (02) 3108410 # 132, Fax: 02 3108416

Abstract: Studies on biodiversity of lichen family usneaceae collected at Phu Hin Rongkla National Park during 2003-2005. They consisted of 1,500 samples. Currently, 2 subgenera, 13 species have been identified. The subgenus *Eumitria* had one species, which was *Usnea baileyi* (Stirt.) Zahlbr. The subgenus *Usnea* had 12 species, namely *Usnea abissinica* Motyka,

U. bornmuelleri Steiner, *U. exasperata* (Müll.Arg.) Motyka, *U. himantodes* Steiner, *U. leprosa* Motyka, *U. maculata* Stirt., *U. nodulosa* Swinscow & Krog, *U. perhispedella* Steiner, *U. roseola* Vain., *U. rubicunda* Stirt., *U. submollis* Steiner and *U. undulata* Stirt. These families are distributed in almost forest types. Most of specimens were found on bark and rock. Seventeen lichen substances were detected by thin layer chromatography. The most common chemical substance is usnic acid.

B1_B0158 BIODIVERSITY OF MICRO-SAXICOLOUS LICHENS AT PHU HIN RONG KLA NATIONAL PARK IN THAILAND.

Chutamat Phraphuchamnong, Nutsurang Homchantara, Piboon Mongkolsuk, and Kawinnat Buarueng
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, Thailand, 10240
e-mail: chutamat_b@hotmail.com

Abstract: Micro-saxicolous lichens are crustose and placoid on rocks at Phu Hin Rong Kla National Park. Lichen sample were collected during January 2003 to November 2005. The total of 402 specimens on 4 kinds of rocks consisted of sandstone, conglomeratic sandstone, conglomerate, and siltstone in 5 forest types; lower montane shrub forest, lower montane oak-pine forest, lower montane oak forest, lower montane rain forest and mixed deciduous forest. The samples were identified into 16 families 23 genera 87 species. Twenty species was expected to be new record for Thailand. An unknown species in family Trichotheliaceae was probably a new species of the world.

B1_B0161 BIODIVERSITY OF LICHEN FAMILY PHYSCIACEAE AT PHU HIN RONGKLA NATIONAL PARK

Sanya Meesim, Piboon Mongkolsuk Natsurang Homchantara Kawinnat Buarueng
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok 10240, Thailand.
E-mail address: meesim_sanya@hotmail.com

Abstract: As collecting lichen family Physciaceae samples at Phu Hin Rongkla National Park of Phisanulok Province from 6 ecoforest types, tropical rain forest, lower montane rain forest, lower montane oak-pine forest, lower montane shrub forest, lower montane oak forest and mixed deciduous forest, during January 2003-December 2006, They are compiled totally 509 samples. Basically of taxonomy identifications are made and found 6 genera, *Buellia Dimelaena, Dimaria, Heterodermia, Phaeophyscia* and *Pyxine*, and 49 species. *Dimelaena thysanota* is a new record of Thailand. Others Heterodermia PH 1, 2, 3, 4, 5, are expected to be new species of the world. The most distribution of lichen species in lower montane rain forest type and the second is in, lower montane oak-pine forest, lower montane shrub forest, lower montane oak forest and tropical rain forest, respectively. The less diversity of lichen is in mixed deciduous forest type. The dominant species of lichen family Physciaceae is *Heterodermia appendiculata*.

B1_B0162 STUDY ON TAXONOMY AND ECOLOGY OF MACROLICHEN AT PHU HIN RONGKLA NATIONAL PARK.

Kawinnat Buaruang, Piboon Mongkolsuk, Natsurang Homchantara and Kajohnsak Vongshewarat
Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamark, Bangkapi, Bangkok, Thailand.
E-mail: b_kawinnat@hotmail.com, Tel: (662)310-8395, Fax: (662)310-8416

Abstract: This study is taxonomy and ecology of epiphytic macrolichens at well-reserved areas of Phu Hin Rongkla National Park. Epiphytic macrolichens are foliose lichens which attach to substrates by rhizine and have 2 types of internal structures: homoiomerous, in which algae and fungi are not stratified, and heteromerous in which algae and fungi are stratified into layers. Photobionts are either green algae or blue green algae. Three hundreds and fifty-one specimens collected in January 2003 - 2005 from 7 forest types were identified into 8 families, 20 genera and 61 species. Sixty-one percent belongs to the family Parmeliaceae, 10% to Collemataceae, 8% to Pannariaceae, 8% to Coccocarpiaceae, 2% to Crocyniaceae, Hypogymniaceae and Phyllopsoraceae. Six species, *Coccocarpia erythrocardia, Collema subnigrescens, Leptogium askotense, Hypotrachyna pseudosimosa, Sticta tomentosa* and *Pannaria aenea* are new records in Thailand. Eleven species are expected to be new species. The areas of lower montane shrub forests had the highest diversity while the tropical rain forests had the lowest diversity.

B1_B0164 Effects of microclimate on survival and growth of lichens after transplantation to host trees in various ecosystems

Mongkol Pangpet and Kansri Boonpragob
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi,
Bangkok 10240, Thailand; e-mail address: gotkol@hotmail.com, Fax: (662)310-8395

Abstract: Lichens have wide range of distribution. They can be found in every type of ecosystems, however, they are relatively restricted to microclimate of particular habitats. This study focuses on the influence of changes in microclimate after transplantation on survival and growth of lichens. Five hundred and fifty-two thalli of *Parmotrema tinctorum* (Despr. ex Nyl.) Hale were transplanted from secondary forest to host trees in four forest types at Khao Yai National Park. The transplantation was performed in October 2003. Thirty-five months after transplantation, 168 thalli (30 %) survived. The highest survival rate was observed in the secondary forest (SF) and subsequently lower in the lower montane rain forest (LMF), the tropical rain forest (TRF) and the dry evergreen forest (DEF) with the rates of 52, 50, 39, and 38 % respectively. The highest average growth rate of 0.4 mm/month was found in the LMF, and lower in the SF, TRF and DEF measured 0.28, 0.26 and 0.1 mm/

month respectively. The maximum growth rate, observable in LMF, was 0.95 mm/month. Lichens transplanted to SF and LMF tend to have higher survival rates than those in TRF and DEF. Light intensity was crucial for survival and establishment of the transplanted lichens. The lowest survival rates were found on lichens transplanted to tree bases in LMF, TRF and DEF, where light intensity during the day averaged as low as 6, 7 and 24 μmol $m^{-2}s^{-1}$ respectively. The areas where lichens grew relatively well were from the mid-trunks to the canopies, where light intensities ranged from 20 to 200 μmol $m^{-2}s^{-1}$. The most successful lichen transplantation was observed in the secondary forest because the trunks of the host trees were well illuminated with 200 μmol $m^{-2}s^{-1}$ light intensity.

B1_B0165 Phenology and pollen tube development of *Kaempferia parviflora* Wall ex Baker, Zingiberaceae)

PIYARAT ITHARAT
Biology Department, Faculty of Science, Khon Kean University, Khon Kean.

Absract: Phenological study of *Kaempfena parviflora* Wall ex Baker was conducted for 5 months from the beginning of May to October 2004. The first leaf is developed in 4-7 days after growing and becomes mature in 8-11 days. The second and the third leaves become fully developed in 30 and 50 days after propagation respectively. The inflorescence consists of 22-27 flowers, and starts blooming in 45-60 days after propagation. The flowering period is range from 24 to 32 days. The receptive flower opens from 5-9 am. If no pollination occurs within this period, the flower can extend the opening period up to 6 hours before senescence. The petals of pollinated flower are senesce within 2-3 hours after pollination. The clearing technique with aniline blue straining is used for the pollen tubes activity on the stigma observation. The germinating of pollen tubes from the tip of stigma to the ovules at the base of ovary takes about 24-36 hours after pollination.

B1_B0167 Genetic comparison of the white shrimp grown at different growth rate (*Litopenaeus vannamai*) using Amplified Fragment Length Polymorphism (AFLP) technique

Wikit Phinrub[1,2], Pluang Suwanmanee[1], Jamjun Pechsiri[1], Tanapan Phattamarnon[1]
[1] Faculty of Science Thaksin University, Songkhla 90000 Thailand Tel : 66-74-318555 Fax : 66-74-44-3966
[2] Faculty of Science and Fishery Technology, Rajamangala University of Technology Srivijaya Trang campus, Sikao, Trang 92150 Thailand. Tel : 66-75-274151-8 Fax : 66-75-274159
E-mail: wikit_ph@hotmail.com

Abstract: White shrimp (*Litopenaeus vannamei*) is the important commercial aquatic animals in Thailand. The broodstock have been illegally imported by some hatchery, resulting in low quality of post larva. In this study, genetic variation of white shrimps undergo different growth from the same ponds were investigated using amplified fragment length polymorphism (AFLP). One hundred and fifty individual small shrimps (3-5 grams) and large shrimps (13-19 grams) were selected from 3 ponds. Caudal fin clips were isolated DNA for AFLP analysis. Two out of ten primer-pairs produced a total of 148 fragments. One hundred and twenty one bands (81.75%) were polymorphic. Genetic distance estimates based on AFLPs was calculated and found to range from 0.318 to 0.744. An unweighted pair group method of arithmetic average (UPGMA) dendrogram clearly separated the small shrimp from the large shrimp. The result indicated that the different growth of the white shrimp may be related to genetic variation.

B1_B0175 Transplantation of lichen with isidia of *Parmotrema tinctorum* and soredia of *P. praesorediosum* on tree barks at Khao Yai National Park, Thailand

Mongkol Pangpet and Kansri Boonpragob
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok 10240, Thailand;
e-mail address: golkol@hotmail.com, Fax: (662)310-8395

Abstract: Lichens can be regenerated and distributed by means of vegetative structures, isidia and soredia. However, successful establishment depends on microhabitats and microclimate. The objectives of this study are 1) to explore the possibility to propagate lichens by using vegetative propagules, isidia and soredia, from *Parmotrema tinctorum* (Despr. ex Nyl.) Hale and *P. praesorediosum* (Nyl.) Hale and 2) to study the effects of microclimate on establishment and growth of the transplanted isidia and soredia. The study was performed by using five hundred and eighty samples of soredia and isidia, each from the lichens grew in the secondary forest. They were transplanted to 3 host trees in 4 different forest ecosystems at Khao Yai National Park in October 2003, and last to until present date. After 34 months, 49 isidia and 14 soredia developed into small thalli. The isidia showed higher ability to adapt and grew in the new environment better than the soredia. However, microclimate of the new habitats influenced the establishment and growth of these vegetative propagules differently. The most successful development of juvenile lobes was observed in the secondary forest, where 33 isidia and 9 soredia developed into thalli. Those transplanted to the dry evergreen forest had thalli developed from 9 isidia and 2 soredia and tropical rain forest from 7 isidia and 3 soredia. In the lower montane rain forest, the samples were able to grow in the first 12 months prior to be grown over by the algae *Trentepohlia*. The tree trunk in the secondary forest received an average intensity of light averaged 308 μmol $m^{-2}s^{-1}$ through all levels of the trunks, which seems to be enough for growth. In the dry evergreen forest and the tropical rain forest, successful establishment and growth were generally found from mid-trunk to canopy. These levels were illuminated sufficiently in early morning, when the surroundings were moist.

B1_B0176 DIVERSITY AND NUTRITIONAL VALUES OF EDIBLE AQUATIC INSECTS IN BAN THI AND MUEANG LAMPHUN DISTRICT, LAMPHUN PROVINCE

Waraporn Noikong[1,2] and Chitchol Palarak[2]
[1] Department of Applied Biology, Faculty of Science, Rajabhat Piboonsongkram University, Pitsanulok, Thailand
[2] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Abstract: Diversity and nutritional values of edible aquatic insects were investigated in the areas of Ban Thi and Mueang Lamphun districts, Lamphun province during November 2004 to November 2005. Samples of edible aquatic insects were collected from 6 sampling sites and 6 local markets. In addition, physical and chemical parameters of water quality were also determined. The result showed that total edible aquatic insects were 3 orders 10 families and 20 genera. Family Notonectidae represented as highest number of individual. According to the diversity index, Ban Jam, was found as the highest diversity (2.98) while Ban San Rim Ping, showed highest species richness (3.14). For nutritional values analysis, Family Hydrophilidae in order Coleoptera showed highest protein content, Family Belostomatidae in order Hemiptera showed highest crude fat level whereas, highest level of carbohydrate, fiber and ash were belonged to Family Gomphidae in order Odonata. The physical and chemical parameters analysis revealed that there were no significant different among sampling sites and water quality assessment resulting as mesotrophic - eutrophic status The application of Multivariate Statistical Package (MVSP) for data analysis indicated as followings: based on biological parameter, MVSP can cluster the samplings sites into 5 groups whereas 4 groups of sampling sites were generated based on physical and chemical parameters.

B1_B0181 ECOLOGICAL STRATEGIES OF EPIPHYTIC LICHEN COMMUNITIES IN THE TROPICAL RAIN FOREST AT KHAO YAI NATIONAL PARK, THAILAND

Wetchasart Polyiam and Kansri Boonpragob
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, Thailand
Tel: 02 3108395 Fax: 02 3108416, E-mail: wetchsart@hotmail.com

Abstract: This study explored species composition of lichens on tree trunks of *Castanopsis acuminatissima* (Blume) A. DC and *Dipterocarpus* gracilis Blume grew in the tropical rain forest at Khao Yai National Park. The objectives were to compare lichen communities on microhabitats along environmental gradients from bases to canopies and on four compass directions of the two different host trees. Environmental factors that governed distribution and biodiversity of lichens will be assessed in order to identify ecological species group of lichens that can be used as bioindicator of environmental quality. The study was performed on 10x50 cm quadrats on three trees of each host species at the base, middle and canopy of the trunks, as well as on the N, S, and W aspects at the middle of the trunks. A total of 270 species were identified, of which only 31 species (or 11%) inhabited both host species, whereas 153 species (57%) preferred *C. acuminatissima*, and 86 species (32%) harbored only *D. gracilis*. Over half of the total taxa found less than two thalli. Beta diversity values and Canonical Correspondence Analysis (CCA) indicated that host species was the most important factor, and vertical stratification contributed the second, that coursed the differences in lichen communities. Lichens on four compass directions were less different. The east facing trunks had the richest number of species, but the northern aspects show greatest differences in taxa. Detrended Correspondence Analysis (DCA) segregated all lichens into nine ecological groups mediated through microclimate and substrate. These were groups of indicator species that can be used for monitoring site quality. In addition, this study underpins the importance of heterogeneity of host tree, and microclimate along vertical strata as the crucial factors for making up rich lichen flora.

B1_B0182 MONITORING GROWTH AND LIFE LONGEVITY OF LICHENS AT KHAO YAI NATIONAL PARK, THAILAND

Wetchasart Polyiam, Wasana Cheusook, and Kansri Boonpragob
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, Thailand
Tel: 02 3108395 Fax: 02 3108416, E-mail: wetchsart@hotmail.com

Abstract: This study aimed at comparing growth and longevity of tropical lichens in six forests. Growth of tropical lichens is largely unknown. The information can be used as baseline data for conservation and sustainable utilization of lichen resources. Measuring expansions of 306 lichen thalli were performed during 1999-2005. Thallus growths were obtained by tracing their edges over transparent sheets at different time intervals. Growth rates were expressed as increasing thallus diameters per unit time. After 7 years, 57 % of the thalli disintegrated, mostly due to natural factors, only 132 thalli remained intact. Regardless of the lower montane forest, the highest rate of survival among the crustose thalli was found in the tree plantation (TP), and subsequently lower in the dry evergreen forest (DEF), the dry dipterocarp forest (DDF), the tropical rain forest (TRF) and the secondary forest (SF) accounted for 82, 71, 67, 49 and 44 % respectively. The foliose lichen had the highest survival rate in the DDF and lower in the TP, LMF, SF and DEF recorded 55, 33, 21, 18 and 13 % respectively. All of them in the TRF disintegrated. The remaining crustose thalli had average growth rate of 2.2 mm/year (n = 88), whilst 44 of the survival foliose thalli grew 5.2 mm/year. The highest growth rate of 8 mm/year was measured from the SF for the crustose lichens, and the 12.3 mm/year for the foliose lichens from the LMF. This investigation reveals that life longevity of the foliose lichens in the wet conditions of the TRF, DEF and SF seems to be shorter than those inhabited the dry site of the DDF, although growth rate of the former group was higher.

B1_B0189 GROWTH AND ANTIMICROBIAL ACTIVITY OF SOME LICHEN MYCOBIONT FROM THAILAND

Montri Sanglarpcharonekit and Ek Sangvichien
Department of Biology, Faculty of Science, Ramkhamhaeng University, Hua Mark, Bangkok 10240, Thailand:
e-mail address: graphids@yahoo.com

Abstract: The aim of this work was to isolate axenic culture of tropical mycobiont (lichenized fungi) components and to observe growth conditions in laboratory. Common mycological media, Chemical Defined Medium (CDM), Corn Meal Agar (CMA), Malt Yeast Extract Agar (MYA), Oat Meal Agar (OMA) and Potato Dextrose Aar (PDA) were used in solid culture for growth rate studied. MYA, OMA, and PDA were proved to be better medium for cultivation of these mycobionts than CMA and CDM. The antimicrobial effects of metabolites produced by cultivation of these mycobionts were also investigated. Culture broth from *Graphina albissima* and *Pyrenula* sp. represented inhibition of E. coli and *P. aeruginosa*.

B1_B0192 EFFECT OF OZONE ON ACTIVE CONSTITUENTS CHANGES OF SOME BEVERAGE HERBS DURING STORAGE

Kanda Whangchai, Chetsada Gonsurin, Peeravut Wongsawad
Department of Biology, Faculty of Science, Chaing Mai University, Chaing Mai, Thailand 50200.
E-mail: kanda l@chiangmai.ac.th, Fax: (66-53)-892259

Abstract: This study was conducted to survey the density of microbial contaminants in beverage herbs which sold in Warorod Market. The samples of rosella, bael fruit, chrysanthemum and lemon glass were evaluated. The results showed that rosella was the most contaminants followed by chrysanthemum, bael fruit and lemon glass respectively. Also the disinfection efficiency of ozone on microbial contamination of these herbs was studied. Ozone fumigation for 5, 10, 15, 30, 60 and 120 min. was done in fumigation chamber. The results showed that ozone fumigation for 60 min. was effective to reduce contaminants. Then the volatile oil contents in chrysanthemum after ozonation was determined by solvent extraction method with dichloromethane. Fumigation with ozone for 60 min. was effective to reduce contaminants. When study about volatile oil content changes during storage of treated herbs. The results revealed that volatile oil content was reduced from 4.51 g. to 3.93 g. when after storage for 1 month. Therefore, packing methods after ozonation to maintain volatile oil contents in some beverage herbs should further investigated.

B1_B0193 GENOTOXICITY OF $CdCl_2$ IN PERIPHERAL ERYTHROCYTES AND GILL EPITHELIAL CELLS OF *Clarias macrocephalus*

Sunate Googsantea, Nongnuch Gumlungpat, Renu Vejaratpimol
Biology Department, Faculty of Science, Silpakorn University, Nakorn Pathom 73000, Thailand

Abstract: Genotoxicity of *Clarias macrocephalus* was used as a bioindicator for the monitor of aquatic pollutants using micronucleus test (MNT). The fish were exposed to 0.01-0.05 mg/L $CdCl_2$ in aquaria for 24 and 48 h (n=10). Three-thousand red blood cells (RBCs) and two-thousand gill epithelial cells (GECs) from each fish were observed for micronuclei (MN) and five nuclear abnormalities. The micronucleus frequencies (MNF) in RBCs were significantly different in $CdCl_2$-treated fish at a 0.04-0.05 mg/L for 24 h and 0.02-0.05 mg/L for 48 h compared to the control group ($p<0.05$). While the MNF in GECs were significantly different in $CdCl_2$-treated fish at a 0.02-0.05 mg/L for 24 h and 0.03 and 0.05 mg/L $CdCl_2$ for 48 h compared to the control group ($p<0.05$). Lobed nuclei frequencies in RBCs were significantly different in $CdCl_2$-treated fish at 0.03-0.05 mg/L for 24 h and 0.01 mg/L $CdCl_2$ for 48 h. While the frequencies of lobed nuclei in GECs were significantly different in $CdCl_2$-treated fish at a 0.04-0.05 mg/L for 24 h and 48 h compared to the control group ($p<0$ 05). Vacuolated nuclei frequencies in RBCs were significantly different in $CdCl_2$-treated fish at 0.03 mg/L for 24 h and 48 h. While the frequencies of vacuolated nuclei in GECs were significantly different in $CdCl_2$-treated fish at a 0.03 and 0.05 mg/L for 24 h compared to the control group ($p<0.05$). But the blebbed, notced and binucleated nuclei frequencies in both cell types with all of the doses were not significantly different compared to the control group ($p<0.05$). The results indicated that the MNF obtained from GECs are 2 fold more sensitive than that of RBCs in 24 h $CdCl_2$-treated fish. Thus, the micronucleus test in *C. marcrocephalus* provides adequate sensitive method and this fish can be use as a sentinel detecting the genotoxic levels of $CdCl_2$ contamination in freshwater environment.

B1_B0194 CHARACTERIZATION OF SPONTANEOUS MUTATED HUMAN AMNIOTIC CELL LINES.

Sirimanus Sangngam[1], Kamol Dasa[1], Weerah Wongkham[1], and Umnat Mevatee[2]
[1] Department of Biology, Faculty of Science, Chiang Mai University, Huikaew Road, Chiang Mai, Thailand.
E-mail: sirimanus13@hotmail.com, Fax: (665)3892-259
[2] Department of Anatomy, Faculty of Medicine, Chiang Mai University, Huikaew Road, Chiang Mai, Thailand.

Abstract: This research aim to study the spontaneous mutation and the biology of the human amniotic cells *in vitro*. The amniotic fluid was randomly taken case by case as sample provided from the Human Genetic Laboratory, Faculty of Medicine, Chiang Mai University. Only 2 from 120 samples exhibited the continuous cell lines *in vitro* named as AMC-K46 with XY and AC-F2 with XX chromosomes. Three types of cell morphology were observed in the culture of both cell lines; fibroblast-like cells, epithelial-like cells and the bigger-round giant cells with the length 35.0-65.0, 25.0-50.0 and 75.0-110.0 micrometers respectively. The two cell lines expressed similar growth pattern with 8 days to the confluent density of 1.4-1.5 x 10^5 cells/cm^2. The doubling time was 24-48 days with the characteristic of contact inhibition monolayer. The aneuploidy with the mosaicism and chromosome aberrations was the important evidence of the spontaneous mutation of the two cell lines. This two cell lines are apparently the first 2 amniotic cell lines derived from Thais and would be useful in the future research.

B1_B0195 EFFECT OF PIPERINE TO *CAENORHABDITIS ELEGANS* AND THREE HUMAN CANCER CELL LINES; C-32, HELA, AND PRIMARY LUNG CANCER CELL COMPARE WITH NORMAL FIBROBLAST CHICH CELL

Korakot Atianasuppat[1], Weerah Wongkham[1], Puttinan Meepowpan[2], and Prasat Kittakoop[3]
[1] Department of Biology, Faculty of Science, Chiang Mai University, 239 Huay Keew Road, Chiang Mai 50200, Thailand
E-mail: june_chiro@hotmail.com, Fax: (665)3892-259
[2] Department of Chemistry Faculty of Science, Chiang Mai University, 239 Huay Keew Road, Chiang Mai 50200, Thailand
[3] Chulabhorn Research Institute, Vibhavadee-Rangsit Highway, Bangkok 10210, Thailand

Abstract: Piperine, an alkaloid amide isolated from *Piper*, was studied for their anti-nematode activity against *Caenorhabditis* elegans and anticancer against; amelanotic melanoma (C-32), cervical carcinoma (HeLa), and a primary lung cancer cells. Positive controls were studied on praziquentel and ellipticine in accordance to the anti-nematode and anticancer activities respectively. The LC_{50} of piperine to the nematode is 2.31×10^2 µg/ml, higher effective than that of praziquentel by half. Moreover, piperine is also more selective to kill *C. elegans* than praziquentel with the selective index of 0.2. But in anticancer activity, LC_{50} of piperine are 3.52×10^3, 3.61×10^5, and 2.63×10^3 µg/ml for C-32, HeLa, and the primary lung cancer cells, respectively. In addition, piperine is less selective to kill the three cancer cell lines than ellipticine. The results in this study clearly indicated that piperine expressed strong anti-nematode activity but without anticancer activity (with selective index > 1).

B1_B0198 Lankesterella IN RICE-FIELD FROG, *Hoplobatrachus rugulosus* AND ITS INFECTION IN GLOSSIPHONIID LEECH

Nitchatorn Sungsirin, Malinee Chutmongkonkul and Putsatee Pariyanonth
Department of Biology, Faculty of Sciences, Chulalongkorn University, Bangkok, 10330, Thailand; Tel: (66 2) 2185265

Abstract: During March to August 2006, the infection of *Lankesterella* in wild rice-field frogs, *Hoplobatrachus rugulosus*, was investigated in 4 provinces of Thailand, Ubon Ratchathanee (number=49), Nakorn Ratchaseema (number=33), Surin (number=14) and Chiang Mai (number=9). These prevalences of *Lankesterella* were 55.10, 3.03, 28.57 and 55.56% and the parasitaemias were 0.06, 0.01, 0.07 and 0.12 %, respectively. The parasite was transmitted to laboratory-reared glossiphoniid leeches through the blood feeding of naturally infected rice-field frog, *Hoplobatrachus rugulosus*. Sporozoites were observed in salivary gland of the experimentally infected glossiphoniid leeches 28, 35, 42 and 49 days after blood feeding. The result showed that *Lankesterella* in glossiphoniid leeches had moved from gut into salivary gland by the time at least 28 days.

B1_B0200 SOME FEEDING PLANTS OF DUSKY LANGUR (TRACHYPITHECUS OBSCURUS FLAVICAUDA) AT KHAO LOMMUAK, PRACHUAP KHIRI KHAN, THAILAND.

Nantiya Aggimarangsee
Department of Biology, Faculty of Science, Chiang Mai University, Thailand.
E-mail: scboi006@chiangmai.ac.th and ooichoon@yahoo.com

Abstract: Dusky langur (*Trachypithecus obscurus flavicauda*) is one of the protected wildlife species of Thailand. It can be found in forest ranges from Phetchburi Province down to the Peninsula Malayu. The study of behavior and ecology of the dusky langur at Khao Lommuak in Prachuap Khiri Khan Province during 2001-2002 discovered that the langur at this site consumed both natural and provisioning food. Although, the langur was over-fed by humans but natural food was its major food supply (77%). Its natural food comprised leaves, flowers, fruits and other plant parts of about 40 plant species. Percentage of plant-part consumption comprised young leaves (38%), flowers (26%), young fruits (20%) and other plant-parts such as mature leaves, ripe fruits, bark and seeds (16%). The result showed that although the langur was over-fed by human but natural plants are considerably needed.

B1_B0205 MOLECULAR PHYLOGENETIC RELATIONSHIPS OF SOME *SALACCA* IN THAILAND, MALAYSIA AND INDONESIA

Achariya Rangsiruji, Tapawittra Pongpawe and Thawat Donsakul
Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

Abstract: Salacca, a genus of Arecaceae (Palmae), consists of 21 species which are distributed in southeast Asia. Classification of plants in this genus is complicated due to similar morphological characters. Our molecular phylogenetic study of these plants included 12 taxa from Thailand, Malaysia and Indonesia. Based on ITS analyses by neighbor joining and maximum likelihood methods, results revealed four important clades. By using *Eleiodoxa conferta* as an outgroup, **clade A** (Rakam, Sala-Sainampeung, Rakam-Rainam and Sala-Mho), **clade B** (Sala-Nernwong and Sala-Sumalee), **clade C** (*S. ramosiana, S. affinis* and *S. rupicola*) and **clade D** (Salak-Pondoh and Salak-Bali) were obtained. These results supported previous cytogenetic data which classified Thai and Indonesian groups. All plants under study (clade A, clade B and clade D) had the same diploid chromosome number of 28. However, only the plants in the Indonesian group (clade D) possessed satellited chromosomes. These data could serve as a basis for plant selection and breeding.

B1_B0206 KARYOTYPES OF TWO CHANNID FISHES (FAMILY CHANNIDAE): *CHANNA MARULOIDES* AND *C. ASIATICA*

Wichian Magtoon, Thawat Donsakul and Achariya Rangsiruji
Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

Abstract: The purpose of this research was to examine the karyotypes of Channa maruloides and C. asiatica. Results obtained were as follows. The diploid chromosome number of C. maruloides was 2n = 38 and its karyptype comprised 1 pair of metacentric, 1 pair of submetacentric, 1 pair of subtelocentric and 16 pairs of acrocentric chromosomes. The arm number was 42. The diploid chromosome number of C. asiatica was 2n = 44. The karyotype comprised 2 pairs of metacentric, 2 pairs of submetacentric and 18 pairs of acrocentric chromosomes. The arm number was 52. These cytogenetic data can be used for further studies in cytotaxonomy and evolutionary relationships of fishes.

B1_B0207 KARYOTYPES OF FOUR CYPRINID FISHES (FAMILY CYPRINIDAE) FROM THAILAND

Thawat Donsakul, Wichian Magtoon and Achariya Rangsiruji
Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

Abstract: The purpose of this research was to examine karyotypes of 4 cyprinid fishes from Thailand. Results obtained revealed that *Neolissocheilus soroides* had a diploid chromosome number of 98 whereas *N. stracheyi*, *Tor douronensis* and *Cyprinus carpio* had identical number of chromosome with one extra pair (2n = 100). Their karyotypes are as follows. *N. soroides* had 22 pairs of meta-, 8 pairs of submeta-, 2 pairs of subtelo- and 17 pairs of acrocentric chromosomes. *N. stracheyi* had 15 pairs of meta-, 9 pairs of submeta-, 3 pairs of subtelo- and 23 pairs of acrocentric chromosomes. *T. douronensis* had 10 pairs of meta-, 14 pairs of submeta-, 9 pairs of subtelo- and 17 pairs of acrocentric chromosomes. *C. carpio* had 10 pairs of meta-, 11 pairs of submeta-, 4 pairs of subtelo- and 25 pairs of acrocentric chromosomes. *N. soroides*, *N. stracheyi*, *T. douronensis* and *C. carpio* had arm numbers of chromosomes equaled 158, 148, 148 and 142, respectively. These cytogenetic data can be used for further studies in cytotaxonomy and evolutionary relationships of fishes.

B1_B0208 ANATOMICAL STUDY OF SOME AQUATIC PLANTS IN NONGHARN KUMPAWAPEE UDON THANI PROVINCE

Tasanai Punjansing[1] and Seewapong Chamratpan[2]
[1] Science Center, Udonthani Rajabhat University, 64 Taharn Road, Udonthani, Thailand.
E-mail:cartoon_pine@hotmail.com, Fax: (664)224-1418
[2] Department of Biology, Faculty of Science, Udonthani Rajabhat University, 64 Taharn Road, Udonthani, Thailand.
E-mail: s_chamratpan@yahoo.com, Fax: (664)224-1418

Abstract: Aquatic plant life cycles are in water at least short a period of time. Thirteen species of aquatic plants collected from Nongharn Kumpawapee, Udonthani, and Anatomical characteristic of stem x-section and epidermis were studied. X-sections of stem showed plenty of air spaces.These characteristics showed they needed more oxygen for respiration. Xylem tissues were not well developed. Pith cavity was found in *Persicaria attenuata* and *Commelina diffusa*. Epidermis of these plants were scattered with stoma both sides of leaves. Cuticle layer was thin. These characteristics could be explained why they suddenly wilt when we picked them up. Three stomata types were found. Amonocytic was found in *Sphaerostepharos polycarpus*, *Limnophila* sp., *Ludwigia adscendens* and *Marsilea crenata*. Actinocytic type was in *Commelina diffusa* epidermis. Paracytic stomata type was in *Neptunia oleracea*, *Limnocharis flava*, *Monochoria elata* and *Sagittaria sagitifolia* epidermis.

B1_B0209 Tissue culture of *Thyrsostachys siamensis* Gamble by multiple shoot induction

Apisak Duangmanee, Chockpisit Thepsithar and Aree Thongpukdee
Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand

Abstract: Nodal explants of *Thyrsostachys siamensis* Gamble were cultured on solid Murashige and Skoog's (MS) medium to induce shoots from axillary buds. Benzyl adenine (BA) at concentration of 11.25 µM significantly induced 13.3 shoots per axillary bud. For multiple shoot induction, a cluster of 3 shoots derived from axillary buds was cultured in agitated liquid MS medium supplemented with BA and kinetin (Kn). The combination of 44.40 µM BA and 2.32 µM Kn provided the best multiple shoots with 36.1 shoots within 8 weeks. The multiplication rate was 12 fold. Numbers of multiple shoots induced from stationary and agitated liquid medium were significantly different within 8 weeks of culturing. Root initiation was induced in a cluster of 3-5 shoots on MS medium added with 26.85 µM of naphthaleneacetic acid (NAA) for 3 weeks, and then transferred to MS medium without plant growth regulator for root elongation.

B2_B0002 *GFP-BASED TRACING* METHOD FOR *LACTOBACILLUS PLANTARUM* N014

Parichat Phumkhachorn[1], Pongsak Rattanachaikunsopon[2], Sumpars Khunsook[1]
[1] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
[2] Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand.
E-mail: pongsak@sci.ubu.ac.th, Fax: (045)288380

Abstract: *Lactobacillus plantarum* N014 is a bacteriocin-producing lactic acid bacteria originally isolated from nham. In the development of *L. plantarum* N014 to be used as a starter culture in nham fermentation, a rapid and efficient tracing method of the bacteria is needed in order to monitor its viability and activities during the course of nham fermentation.

In this study, pN014-GFP, a novel plasmid carrying gfp gene was constructed by placing the gene under the control of lactate dehydrogenase promoter (pldhL). The newly constructed plasmid was introduced into *L. plantarum* N014 to give rise *L. plantarum* N01-GFP+ which can be traced directly by using a UV transilluminator or a fluorescence microscope.

B2_B0003 PLASMID VECTOR, pOri253, FOR EXPRESSION OF CHITINASE-ENCODING GENE FROM *BACILLUS CIRCULANS* IN LACTIC ACID BACTERIA

Kittisak chawawisit, Monthon Lertcanawanichakul
School of Allied Health Sciences and Public Health, Walailak University, Thasala District., Nakhon Si Thammarat 80160, Thailand

Abstract: A Chitinase gene from the Gram-positive bacterium *Bacillus circulans* No. 4.1 was cloned in *Enterococcus faecalis* JCM8726 and in the *Lactococcus lactis* L14, isolated from fermented pork. The chitinase gene was not expressed as an active enzyme in both of strains. Moreover, *L. lactis*, contain several native plasmids in theirs cell, showed the plasmid incompatibility. The chitinase gene expression in the Enterococci strain was not due to plasmid deletion, because the isolated plasmid from them showed normal chitinase activity when retransformed into *Escherichia coli* DH5α. It might be caused by regulatory processing in differ cells, such as promoter, Shine-Dalgano (SD) sequence, codon usage, etc.

B2_B0006 Optimization of Pectinases Production from *Paenibacillus polymyxa* N10

Chawanit Sittidilokratna[1], Pilanee Vaithanomsat[1], Surang Suthirawut[2] and Prisnar Siriacha[1]
[1] Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) Kasetsart University, 50 Chatuchak, Bangkok 10900, Thailand.
[2] Department of Microbiology, Faculty of Science, Kasetsart University, 50 Chatuchak, Bangkok 10900, Thailand.

Abstract: Production condition of pectinase enzyme from selected bacterial strains N10, isolated from paper mulberry bark, was optimized. This selected strain was later identified as Paenibacillus polymyxa N10. The optimal cultivating condition to achieve maximum pectinase production included growing the bacteria in basal medium pH 8.0 at room temperature for 3 days with shaking. The characteristics of pectinase in form of pectate lyase was studied. It demonstrated that the most optimum condition for enzyme activation was in 0.1 M ammonium chloride-ammonium hydroxide buffer pH 10.0 at 35 °C. The stability of the enzyme widely ranged between pH 3.0 and 12.0 at room temperature and at temperature from 20 °C to 40 °C for 24 hr. When the crude pectinase was subjected to freeze drying for 24 hr., the enzyme powder was obtained and showed higher activity than crude pectinase in liquid form for 4,000 times.

B2_B0010 RAPID ALCOHOL PRODUCTION FROM MOLASSES

Yupakanit Puangwerakul
Faculty of Biotechnology, Rangsit University, Patumthani, Thailand
E-mail: lombiotec@yahoo.com, Fax: (662) 997-2220 ext.3661

Abstract: Alcohol production from molasses in 1,500 liter fermenter by exponential fed batch process prepared 16% invert sugar content in medium and using *Saccharomyces cerevisiae* RSU no.10 1.11×10^8 cells/ml in 10% inoculum size for starter was studied. The result showed that 8.5 % alcohol (V/V) was received in 12 hours of fermentation period. It found the relationship between alcohol content and the highest number of lived yeast cells at 10^9 cells/ml. The scale up of 1,500 liter in two fermenters continuous process produced 8.0 % alcohol was in 8 hours of fermentation period. It showed that the lived yeast cells were 1.53×10^8 cells/ml, productivity 7.9 g/L.h, the fermentation efficiency was 78%. The system was shown to be stable for over a week.

B2_B0016 Black Disease of Fairy Shrimps and Associated Bacteria

Nooduan Muangsan[1], Suwanna Niamsanit[2] and Surasak Siripornadulsil[2]
[1] Faculty of Biology Khonkhen University 40002 Thailand
[2] Faculty of Microbiology Khonkhen University 40002 Thailand

Abstract: Black disease causing the death of fairy shrimp is typically shown by black nodules on several parts of body such as on the thoracic appendage, antennae, and tail. Isolation and identification of potentially pathogenic bacteria from diseased fairy shrimps (*Branchinella thailandensis*) were carried out. All associated bacteria were identified by morphological studies and biochemical tests and they were classified into four groups as follow: *Aeromonas* spp., *Klebsiella pneumoniae*, *Enterobactor* spp. and *Chryseobacterium* spp. These bacteria may be the exact cause of black disease. Verification of Koch's postulate and identification of bacteria isolated from black diseased fairy shrimps by 16S ribosomal DNA sequences will be discussed. Key words: Bacteria, Black disease, Fairy shrimp

B2_B0054 COMPARISON OF DYE INDICATORS IN SELECTIVE ISOLATION OF THERMOTOLERANT ACETIC ACID BACTERIA

Duthathai Fujaroen, Wasu Pathom-aree*
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract: Thermotolerant acetic acid bacteria were isolated from 15 kinds of flower: *Allamanda cathartica* L., *Antigonon leptopus* Hook.t Arn., *Bidens bipinnaata* L., *Brunfelsia hopeana* Benth, *Cassia surattensis* Burm.f., *Ervatamia divaricata*,

Euphorbia milii Desmoul., *Ixora lobbii* Loud., *Jasminum multiflorum, Lantana trifolia* L., *Lonicera japonica* Thun., *Nerin indicum* Mill., *Petrea volubilis* L., *Plumeria acutifolia* and *Zinnia angustifolia* Kunth. using potato medium supplemented with 4% ethanol (v/v) as enrichment medium with three different dyes : 0.0016 % bromocresol green, 0.0016 % bromocresol purple and 0.0016% bromophenol blue. The numbers of successful isolation obtained from each dye were nearly the same. There were no significant differences between the dyes tested ($\alpha > 0.05$). However, 0.003 % bromocresol purple is recommended for use as dye indicator for the isolation of thermotolerant acetic acid bacteria from flowers due to its most easily observed color change. Morphological characteristics and overoxidation reaction revealed that most of thermotolerant isolates were members of the genus *Gluconobacter*

B2_B0076 Screening of the biosurfactants from thermotolerant microorganisms

พนย อัศวรุจานนท์[1], ชจีนาฏ โพธิเวชกุล[2], ดาช์วดาม์ ภาคพ่อเจริญ[3], ไพศาล ขาวสัก[3], เกษร จันทร์ศิริ[3] และโกสุม จันทร์ศิริ[3]
[1] ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ,
[2] ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ,
[3] ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ,

Abstract: Sixty-one pure isolates of thermotolerant microorganisms were segregated from soil and water samples of hot spring resources at Lumpang, Lumpoon, Chiang Mai, Chiang Rai and Mae Hong Son. Each pure isolates was cultured in LB Broth media at 45° for 14-16 hours prior to centrifugation at 3,000 rpm for 15 minutes. Supernatant was collected and proceeding to oil spreading techniques. It was found that 7 isolates containing 2 isolates of *Bacillus megaterium* and each of 1 isolate of *Pseudomanas thermotolerans, Aneuriniacillus thermoaerophilus, B. amyloliquefaciens, B. amliensis* and *Paenibacillus flavisporus* showed dispersion ability on the oil layer. Later, the supernatant was extracted by using hexane and chloroform/methanol/water (65: 25: 4), respectively. Analysis of the purified biosurfactants by the use of TLC, IR and HPLC with surfactin as a standard compound illustrated that it contained the lipoprotein component.

B2_B0078 Biodiversity studies of thermotolerant Bacillus spp. in the hot spring resources from the south of Thailand based on hypervariable region of 16S rDNA.

Arda Pakpitcharoen[1], Montri Yasawong[1], Phirom Singhaphan[1], Phisarn Khawsak[1], Pornpimon Kanjanavas[1], Sunsanee Phasook[1] and Kosum Chansiri[1]
[1] Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University

Abstract: According to the Department of Mineral Resources, there are approximately 112 hot spring water resources in northern, southern, western and central parts of Thailand. Here, thermotolerant bacteria were isolated from soil and water samples of 34 hot spring resources at Chumphon, Ranong, Phang Nga, Krabi, Trang, Phatthalung, Satun, Yala, Nakhon Si Thammarat and Surat Thani. The 48 pure isolates of *Bacillus* sp. were collected and proceeding to DNA extraction prior to PCR amplication using specific primers for *Bacillus* hypervariable (HV) region of 16S rDNA. The PCR product of 340 bp in length was obtained and sequenced by using dideoxy DNA sequencing method. The nucleotide sequences alignment of HV region was achieved by using program Clustal W in comparison to those of other *Bacillus* spp. in GenBank. Phylogenetic tree was constructed based on Bayesian analysis and revealed that four major groups of *Bacillus* namely A, B, C and D showed possibility to be novel species, especially, those in group A. These thermotolerant *Bacillus* spp. have been investigated for their production of some commercially important bioactive compounds such as biocatalysts (enzymes), biosurfactants, anti-microbial agents, anti-cancer agents and anti-aging agents.

B2_B0081 ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF TACCA CHANTRIERI

Manee Tantirungkij[1], Pacharaporn Phupaiboon[2] and Orawan Chountragoon[1]
[1] Central Laboratory and Greenhouse Complex, KURDI, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom 73140
E-mail:rdimat@ku.ac.th, Fax: (034)351-392
[2] Scientific Equipment Center, KURDI, Kasetsart University, Chatuchak, Bangkok 10900

Abstract: Ethanol extract of the rhizomes of *Tacca chantrieri* (EX3) showed the inhibitory effect against the tested bacteria both Gram positive (*Bacillus subtilis and Staphylococcus aureus*) and Gram negative (*Escherichia coli, Enterobacter cloaceae, Aeromonas hydrophila* and *Serratia marcescens*) as well as yeast (*Candida albicans*). However, EX3 at the concentration of 250 mg/ml had no bactericidal effect against *C. albicans*. EX3 was further partitioned into non polar and polar groups by the mixture of ethyl acetate and water (2:1). The non-polar group in the ethyl acetate fraction (EA-EOH8) exhibited the inhibitory and bactericidal at the concentration of 6.25 mg./ml., while the polar group in the water fraction (EA-H2O8) at the concentration of 250 mg./ml. did not show the inhibitory and bactericidal effect against *Bacillus subtilis* and *Serratia* marcescens. Moreover, the extract obtained from boiling the fresh rhizomes in the hot water for 2 hours (DH2) had the *inhibitory* and bactericidal effect against the tested bacteria and yeast except for *Ser. marcescens* and *C. albicans* at the concentration of 160 mg./ml.

B2_B0089 ISOLATION OF LACTIC ACID BACTERIA FOR INHIBITING THE MAJOR ORAL PATHOGEN, *PORPHYROMONAS GINGIVALIS*

Suksangpleng T[1], Sookkhee S[1], Muangmanee M[2], Pattamapun K[3]
[1] Department of Microbiology, Faculty of Medicine; [2]Department of Odontology and Oral Pathology, [3]Department of Restorative Dentistry, Faculty of Dentistry, Chiang Mai University, Thailand. Correspondence

e-mail address: ssookkhee@hotmail.com

Abstract: The aims of the present study were to isolate and characterize the antimicrobial lactobacillus recovered from goat's intestinal and milk samples. Among 32 goats, 1000 lactobacilli isolates were recruited to determine their antimicrobial activities. Three isolates possessed the strongest inhibitions against various bacterial indicators including the periodontal bacteria, *Porphyromonas gingivalis* W50. These isolates were later found to be microaerophilic, Gram-positive, acid-fast negative, non spore-forming, non capsule-forming and catalase-negative bacilli. According to API 50 CHL, the results revealed that these isolates belong to *Lactobacillus paracasei* subsp. *paracasei*. Their bacteriocin activities were significantly declined at pH ≥8.0 or after treating with trypsin and pepsin, and also reduced after heating at ≥80°C. Crude bacteriocins of such isolates were stable and active over a wide pH range of 4-8. It may be suggested that the bacteriocins harvested from these isolates are possibly to characterize, purify and further formulate as the agent for inhibiting P.gingivalis.

B2_B0104 A PRELIMINARY STUDY OF THE BIOLOGICAL CONTROL OF PLANT PATHOGENS, *Phytophthora* spp., BY ANTAGONISTIC BACTERIA

Nirawadee Shreesuvun, Eakachai Pathomsuriyaporn and Eakaphun Bangyeekhun
Department of Microbiology, Faculty of Science, Silpakorn University.

Abstract: *Phytophthora* spp. are the plant pathogens causing diseases in many economic plant species. To overcome these plant pathogens, the biological control by using antagonistic bacteria is an alternative to reduce the use of fungicides in agriculture. In this study, we screened the antagonistic bacteria that can inhibit the growth of *Phytophthora* and found that *Bacillus cereus* MM0508 and Pseudomonas sp. MM0573 showed significant inhibition activity on mycelial growth of *P. palmivora* and *P. parasitca*, respectively. In dual culture, the fungal mycelium were limited in extension and became swollen and roughly. This may due to antibiotic substances produced by the antagonistic bacteria.

B2_B0113 MICROORGANISM INFECTION IN JUVENILE SEA TURTLES THAT HAVE BEEN RAISED IN THE SEA TURTLE CONSERVATION STATION, CHONBURI PROVINCE

Korapan Sawetsuwannakun and T. Veerapraditsin*
Department of Microbiology, Faculty of Science, Silpakorn University, Sanamchan Palace, Nakorn Pathom, Thailand 73000
*Corresponding author, email: suy85@hotmail.co.uk, Fax: (+66) 034-255820

Abstract: Due to dramatically decrease in number, sea turtles have been listed on the Endangered Species Act. In Thailand, the Thai Royal Navy has continuingly carried out the sea turtle conservation campaign in order to increase the number of sea turtles in nature. One problem during the process is that the juvenile sea turtles that have been raised in the conservation station were infected, and some were died from these infections. Investigation in the dead juvenile sea turtles found lesions mostly in the skins, pharynxes, and livers. Isolation of microorganisms from the lesions was on nutrient agar or potatoes dextrose agar with a supplement of 5% NaCl, resulting in at least 10 isolates of bactena, 1 isolate of yeast but no growth of any fungi on the medium. The results from gram staining indicated that these bacteria are either gram positive, such as *staphylococcus* spp. which can be found in all 3 lesions, or gram negative bacteria, and they are in rod, coccus, and oval shapes. Identification of these bacteria will be performed and the obtained information will be used for further study of the causes of diseases in sea turtles.

B2_B0151 *IN VITRO* ANTIFUNGAL ACTIVITY AGAINST PLANT PATHOGENIC FUNGI OF CRUDE EXTRACTS OF SOME LICHENS FROM PHU HIN RONGKLA NATIONAL PARK.

Wonput Hongsachat, Vasan Poengsungnoen and Piboon Mongkolsuk
Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamark, Bangkapi, Bangkok, Thailand.
E-mail: wonput_ja@hotmail.com, wonput_naja@yahoo.co.th, Fax: (662)310-8416

Abstract: The crude extracts from absolute ethanol alcohol of 10 lichen species are tested in vitro for the mycelial growth inhibition of *Colletotrichum gloeosporoides*, causing mango anthracnose, *Colletotrichum piperatum* causing chili anthracnose, *Curvularia eragostidis* causing rusty spot disease of *Dendrobium* cutting flower, *Fusarium moniliforme* causing bakanea disease of rice, *Phytophthora parasitica*, causing stem and root rot disease of citrus, *Pythium diliensis*, causing root rot disease of hog plum and *Sclerotium rolfsii* causing stem rot disease of mung bean. The five concentrations, 1,000 500 100 50 and 10 ppm (part per million), of crude extract were tested. The median effective inhibitory concentration (EC_{50}) against plant pathogenic fungus was calculated using the linear relation between the inhibitory probability and concentration logarithm according to method out line by Finney (1). With highly distinguished to inhibit the mycelial growth of those plant pathogenic fungi, the crude extract of *Canoparmelia owariensis* resulted in the EC_{50} of the above listed pathogens as 51.14, 68.91, 21.29, 66.03, 133.12, 193.05 and 3432.45 µg/ml respectively.

B2_B0156 BIOLOGICAL DEGRADATION OF RICE HUSKS BY CELLULOLYTIC FUNGI

Charida Pukahuta, Jutamas Jitcharoen and Aranya Pimmongkol
Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

Abstract: *Aspergillus* sp., *Trichoderma* spp., *Rhizopus* sp., *Sclerotium* sp. and white rot fungi, *Lentinus* spp. were studied for degradation activity on rice husk substrate. The solid substrate fermentation with 70 % of moisture was enriched with Fahraeus broth. *Lentinus* spp. LP-PT-1, LP-SN-51, LP-SW-3, LP-YD-2, LP-SM-11, LS-BUB-9, LS-BUB-10, LS-KJ-21, KS-

WR, KS-KD performed higher cellulase activity than those of other cellulolytic fungi. Their cellulase activities were 0.068-0.125 U/mg protein while the activities of other cellulolytic fungi were 0.011-0.045 U/mg protein. From the result of comparision between 0.1 % glucose and 0 5 % glucose in rice husk substrates, the 0.5 % glucose increased the cellulase activity of all tested isolates. The cellulase activity of LP-PT-1 showed higher increase upon the addition of 0.5 % glucose. Its activity increased from 0.191 to 0.456 U/mg protein. In solid substrate fermentation of rice husks and submerged fermentation using 2 % rice husks, 0.5 % glucose could increase the cellulase activity in LP-PT-1, LP-SW-3 and LP-SN-51 better than 1% glucose.

B2_B0172 PRELIMINARY STUDY OF ANTIMICROBIAL ACTIVITIES ON MANGOSTEEN

Darawan Thongbutre[1], Suchada Chaisawadi[1], Waraporn Methawiriyasilp[1]
[1] Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand; E-mail:darawan@pdti.kmutt.ac.th

Abstract: To investigate medicinal properties form Thai fruit as natural antimicrobial agents, Mangosteens were selected to screen for antimicobial activities. Fresh extracts and oil extracts of Mangosteen in this study were tested against food poisoning bacteri: *Bacillus cereus, Salmonella typhi* and *Staphylococcus aureus* by using Agar Diffusion Method. Screening for antimicrobial activities of Mangosteens was performed in duplication. Oil extract from the outer peel, the inner peel and flesh of mangosteen showed antimicrobial activities against *Bacillus cereus* and *Staphylococcus aureus*. Only *Salmonella typhi* showed no anti microbial activity. In addition, it was found that mangosteen fresh extract showed no antimicrobial activities, but oil extract showed high activity. The negative control test, 95% ethanol without peel, showed no inhibitory effect on microbial activity. Potential of outer peel, inner peel, and flesh of mangosteen as antimicrobial agent have been concluded. The process development on Mangosteen waste utilization as natural drug has been suggested.

B2_B0174 Screening of Starch Hydrolysis Fungi from Look-pang

Suphaporn Phongmanee and Pantipa Nuamkhuntod
* Department of Food Technology, Faculty of Science, Siam University, Bangkok 10160, Thailand

Abstract: Isolation of fungi from ten sources of Lookpang includings Lookpang of Ampur Chompra Surin province, Ampur Tha-toom Surin province, Petchburi province, Ayutthaya province, Tumbon Kangsanamnang Nakornrachasima province, Roi-Ed province, Ampur Muang Roi-Ed province, Ampur Saylapoom Roi-Ed province, Ampur Muang Prae province and Ubonrachathani province for selection the highest starch hydrolytic activity. Thirty six fungi were isolated. ADM1 coded Fungi which isolated from Lookpang of Roi-Ed province, green mycelium and spores had the highest starch hydrolysis activity on starch agar at 5 days. It showed hydrolytic (clear) zone on starch agar containing of 1% of sticky rice flour and 1% of rice flour at 4.4 and 4.3 centimeter diameter respectively.

B2_B0178 ANTIMICROBIAL ACTIVITIES ON THAI FRUIT SEEDS

Yingsak Kraipinit[1], Surachai Kaewboonruang[1], Patcharin Juntawong[1], Suchada Chaisawadi[1], Darawan Thongbute[1]
[1] Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand; E-mail:kyingsak@yahoo.com

Abstract: To investigate Thai fruit seeds as natural antimicrobial agents, six of fruit seeds including Maprang's seed (*Bouae burmanica Griff.*), Mango's seed (*Mangifera indica Linn.*), Makham's seed (*Tamarindus indica* Linn), Makham pom's seed (*Phyllanthus emblica* Linn), Pomelo's seed (*Citrus maxima Merr*) and Durian's seed (*Durio zibethius Linn*) were selected to screen for antimicrobial activities. Alcohol extraction has been done on all food seed samples. Alcohol extract of all fruit seed in this study were tested against *Bacillus cereus, Salmonella typhi* and *Staphylococcus aureus* by using Agar Diffusion Method. Three of them including Maprang's seed (*Bouae burmanica Griff.*); Mango's seed (*Mangifera indica Linn.*) and Makham pom's seed (*Phyllanthus emblica* Linn); have shown high activities against *Bacillus cereus* and *Staphylococcus aureus*. All of them showed no activity against *Salmonella typhi*. Mango's seed, Maprang's seed and Makham pom's seed exhibited high antimicrobial activities against *Bacillus cereus* and *Staphylococcus aureus*, while mango's seed showed the highest potential activity against both species. The diameter of inhibition zone against B. cereus obtain from activity of mango, maprang and makham pom's seed extracts were 19, 17 and 15 mm. respectively, compared to 17 mm. obtained from inhibition activity of ampicilin. The inhibition against S. aureus were 17, 14 and 13 mm. compared to 34 mm. obtained from inhibition activity of ampicilin. Identification of active compounds and suitable purification method in these fruit seeds have been suggested to further studies. Waste utilization by using fruit seed on high season of the three fruits have been concerned and suggested to further products development.

B2-B0190 GENETIC VARIATION ANALYSIS OF STRAW MUSHROOM VOLVARIELLA SPP. USING THE ITS REGION AND 28S RIBOSOMAL DNA

Thitiyaporn Prayoonmahisorn[1], Ahchara Payapanon[2] and Malee Srisodsuk[3]
[1] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140
[2] Biotechnology Research And Development Office, Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok 10900
[3] Department of Microbiology, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140

Abstract: Volvariella volvacea is an important edible mushroom of the tropics. It is a homothallic species with high genetic variation. Molecular phylogenetic studies of this mushroom will provide basic genetic information for strain improvement. In this study, nucleotide sequence data from the internal transcribed spacer (ITS) and the large subunit ribosomal DNA (28S rDNA) were amplified and analysed from 34 straw mushrooms isolated in Thailand by using specific primer ITS1, ITS4, LROR and LR5. It was found that the ITS and 28S rDNA fragments could not be use for the mushroom strain identification. The different size of 28S rDNA fragments were found to have similar nucleotide sequences while only ITS fragment of No. 22 and 24 exhibited different nucleotide sequence from the others. Physiological characteristics of the mushroom No. 22 and 24 were also different. Therefore, ITS analysis of rDNA may offer the way to improve the straw mushroom for desired properties.

B2_B0191 ITS AND 28S rDNA ANALYSIS OF *AGARICUS* SP.

Suthida Traibut[1] and Malee Srisodsuk[2]
[1] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140
[2] Department of Microbiology, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140

Abstract: *Agaricus bisporus* is one of the most commercially cultivated *Agaricus sp. Agaricus* sp. is also found in Thailand but no commercial production. Because of its good taste, widely consumption and high market value, it is interesting to study the local Agaricus in Thailand for futher commercial production. In this study, tropical Agaricus sp. found in Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom was identified by using morphological characteristics. The mushroom showed white cap with free gill, smooth and blackish-brown spores. The stipe clearly separated from the cap and annulus was present without volva which is an important characteristic of this genus. From internal transcribed spacer (ITS) and large subunit ribosomal DNA (28S rDNA) analysis by using specific primer, ITS1, ITS4, LROR and LR5 as well as phylogenetic tree, it was found that the 713 bps and 747 bps nucleotide sequences from ITS region and 28S rDNA, respectively, were closely related to *Agaricus bitorquis*. The mushroom will be further studied in comparison to the temperate strain.

B3_B0007 Genetic Relationship Analysis of Some *Cucumis sativus* Cultivars Using RAPD Technique

Somrudee Onto, Nittaya Laosat, Wannipa Suksawat, Siam Popluechai, Prapassorn Damrongkool and Ekachai Chukeatirote
School of Science, Mae Fah Luang University, Chiang Rai 57100 Thailand

Abstract: The genetic diversity of some cucumbers, *Cucumis sativus*, was determined by random amplified polymorphic DNA (RAPD) analysis. Twelve lines of *C. sativus* obtained as seeds were grown and their young leaves were then used to extract genomic DNA. To perform the RAPD analysis, forty random primers were used and the results were interpreted based on total and polymorphic scores. In addition, a dendogram was constructed using the UPGMA analysis to trace their phylogenetic relationship. According to the results, these *C. sativus* species examined could be separated into two major subgroups in which the similarity values were between 0.67 – 0.93.

B3_B0023 Nucleotide sequence of the 26S rRNA gene in *Melientha suavis*, and its phylogenetic analysis

Narongchai Tongyoo, Kanya Kawin
Nakhon Sawan Rajabhat University, Nakhon Sawan, Thaialnd. 60000

Abstract: Recently, the nucleotide sequences of 26S rRNA gene were determined from representatives from monocots to eudicots. However, the sequence data of rRNA genes for various plants are still not well established. Here we present the nucleotide sequence of the 26S rRNA gene of *Melientha suavis*, a tree commonly found in mixed deciduous forest and a member of the family Opiliaceae. By using PCR based and DNA sequencing method, we were able to obtain 1,509 bp of the 26S rRNA gene sequence (partial sequence) of *M. suavis* and used for searching in GenBank database. The result showed that the 26S rRNA gene of M. suavis was 96% sequence identity to the corresponding gene of *Opilia amentacea* in the nowadays GenBank database. The 26S rRNA gene sequence of *M. suavis* was aligned by the multisequence alignment program CLUSTAL X Phylogenetic relationships was inferred by using the PHYLIP program. A rooted tree was constructed by distance based, Neighbor-Joining method and drawn with TREEVIEW software. Phylogenetic analysis of *M. suavis* based on its 26S rRNA gene sequence, from the results, indicates that *M. suavis* is related to *O. amentacea* in family Opiliaceae.

B3_B0046 G6PD VIANGCHAN (871G>A) IS THE MOST COMMON G6PD DEFICIENT VARIANT IN THE CAMBODIAN POPULATION

Chalisa Louicharoen[1] and Issarang Nuchprayoon[2]
[1] Biomedical science program, Graduate school, Chulalongkorn University, Rama IV Road, Bangkok, Thailand. E-mail:Chalisa_loui@yahoo.com, Fax: (662)652-4204
[2] Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzymopathy among Southeast Asians. We studied G6PD mutations in 108 migrant Cambodian laborers in Chanthaburi province and cord blood samples from 107 Cambodian newborns at Buriram hospital. Thirty-one (26.1%) of 119 Cambodian males and 3 of 96 (3.1 %) females were G6PD deficient and were assayed for G6PD mutations. G6PD Viangchan (871G>A) was identified in most G6PD deficient Cambodians (28 of 34; 82.4%), G6PD Union (1360C>T) and G6PD Coimbra (592C>T) was found in 1 case each. We concluded that G6PD Viangchan (871G>A) was the most common mutation among Cambodians, which was similar to G6PD-deficiency in Thais and Laotians. This finding suggested a common ancestor among people from there three

countries.

B3_B0060 A NONDESTRUCTIVE, RAPID AND INEXPENSIVE METHOD TO EXTRACT MITOCHONDRIAL DNA FROM NATIVE DUCKS.

Piyanan Leekaew[1], Thaweesak Songserm[2], Apassara Choothesa[1], and Ukadej Boonyaprakob[1]

[1] Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand 10900. E-mail: g4772010@ku.ac.th, Fax: (662)579-7538

[2] Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University Kamphaengsaen Campus, Nakornpathom, Thailand 73140.

B3

Abstract: A low-cost method was developed for the preparation of duck mitochondrial DNA (mtDNA) from a single plucked feather. The procedure was based on alkaline lysis and neutralization. The extract was used directly in PCR (method 1; n = 17) or phenol/chloroform purification of DNA prior to PCR amplification (method 2; n = 20). The quality of the DNA extracted was tested by PCR amplification of a 710-bp fragment of the mitochondrial control region and the PCR products were determined by sequencing analysis. Using this method, duck mtDNA extraction could be performed within 20 min (method 1) or 2 hr (method 2), all of the samples (n=37) yielded amplifiable mtDNA and at least seven-hundred fold saver than proteinase K digestion or commercial kits. The results were reliable and reproducible. This study offers the simple and inexpensive method with no hazardous extraction step for preparing mtDNA from native ducks without harming the birds.

B3_B0062 POPULATION GENETIC STUDIES OF THE BLACK TIGER SHRIMP (*Penaeus monodon*) IN THAILAND BASED ON MITOCHONDRIAL COI SEQUENCES

Bavornlak Khamnamtong[1,2], Sirawut Klinbunga[1,2], and Piamsak Menasveta[3]

[1] Center of Excellence for Marine Biotechnology, [3]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

[2] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani, 12120, Thailand. E-mail: sirawut@biotec.or.th

Abstract: Genetic diversity and population differentiation of *Penaeus monodon* in Thai waters was examined by polymorphism of COI sequences. Initially, the COI-COII gene segment was amplified from representative individuals of *P. monodon*. The amplification product was approximately 1550 bp in length. These gene segments were cloned and sequenced for both directions. Limited sequence divergence was found in COII whereas COI exhibited much greater polymorphism. Primers designed from COI consistently generated the amplification product of 614 bp (hereafter called COI_{614}). The PCR product from 100 individuals of wild *P. monodon* was unidirectional sequenced. The percentage of nucleotide divergences between pairs of sequences was 0.00 - 23.65% whereas that of T17 and other sequences was 37.43 – 55.87%. Forty-three haplotypes were observed. A UPGMA dendrogram indicated three lineages of Thai *P. monodon*. Large nucleotide divergence was observed between inter-lineage haplotypes but limited divergence was found between intra-lineage haplotypes. Distribution frequencies of haplotype clusters indicating the existence of population subdivisions of *P. monodon* based on COI polymorphism ($P < 0.05$). Rapid genotyping of broodstock used for genetic improvement and/or stock enhancement programs can be carried out by DNA barcodes from COI_{614}.

B3_B0064 GENETIC DIVERSITY AND GENOTYPING OF THE BLACK TIGER SHRIMP (*Penaeus monodon*) IN THAILAND BY PCR-RFLP AND PCR-SSCP ANALYSIS OF 16S rDNA AND COI

Bavornlak Khamnamtong[1,2], Sayan Prakobpetch[1,2], Sirawut Klinbunga[1,2], and Piamsak Menasveta[3]

[1] Center of Excellence for Marine Biotechnology, [3]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

[2] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani, 12120, Thailand. E-mail: sirawut@biotec.or.th Fax: 0-2254-7680

Abstract: Genomic DNA of *P. monodon* was amplified using universal primers of 16S rDNA and COI-COII. The amplification product was approximately 560 bp and 1550 bp in length, respectively. These gene segments were cloned and sequenced. More specific primers for each gene were designed and consistently generated the amplification product of 312 bp (16S $rDNA_{312}$) and 614 bp (COI_{614}), respectively. PCR-RFLP of 16S $rDNA_{312}$ with *Alu* I, Ssp I and Vsp I was applied for examination of genetic diversity in natural *P. monodon* (N = 86). Only 3 mitotypes; AAA, ABA and ABB which were distributed in 51.2%, 43.0% and 5.8% of investigated specimens were observed. This implied low polymorphism in this gene region and/or limited sensitivity of PCR-RFLP. SSCP analysis was then applied to determined genetic diversity of this gene segment using a relatively large sample size (N = 185) from geographically different locations in Thai waters. A total of 17 SSCP patterns were found. The average number of individuals that possibly share identical SSCP patterns was 10.88. The genetic distance between pairs of geographic samples was comparatively low in all geographic samples (0.0946 - 0.0996). Results also indicated that PCR-SSCP is more sensitive than PCR-RFLP when the same DNA segment was analyzed. In addition, the COI_{614} gene segment of the same sample set (N = 136) was also analyzed by PCR-SSCP. A total of 38 SSCP genotypes and high genetic diversity between pairs of geographic sample (0.0691 - 0.9125) were illustrated. The average number of individuals that possibly share identical SSCP patterns was 3.579. The ratio between the number of genotypes and the number of investigated specimens for PCR-SSCP of 16S $rDNA_{312}$ and COI_{614} was 0.092 and 0.279 indicating that COI display

greater polymorphism than did 16S rDNA. A large number of SSCP genotypes found from SSCP analysis of 16S $rDNA_{312}$ and COI_{614} suggested the possible application of this simple technique for rapid genotyping of domesticated stocks of *P. monodon*.

B3_B0068 MUTATION IN α-GLUCOSIDASE GENE AND RESISTANCE AGAINST *BACILLUS SPHAERICUS* STRAIN 2362 OF *CULEX QUINQUEFASCIATUS* IN THAILAND

Mongkon Audtho[1], Monrudee Ratlerdkarn[1], Boonhiang Promdonkoy[1] and Usavadee Thavara[2]

[1] Biotec Central Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Phaholyothin Rd., Klong Luang, Pathumthani, Thailand.
E-mail: mongkon@biotec.or.th

[2] Division of Medical Entomology, Department of Medical Sciences, NIH, Ministry of Public Health, Nonthaburi, Thailand.

Abstract: Application of *Bacillus sphaericus* strain 2362 to control nuisance mosquitoes (*Culex quinquefasciatus*) in low-income communities in Thailand rapidly triggered higher than 100,000-fold resistance of the mosquitoes to the bacteria. The resistance is believed to be an alteration in binding between binary toxin from B. sphaericus and α-glucosidase in the mosquito guts. In this study, DNA sequence of α-glucosidase gene, which encodes for 580 amino acids, was cloned from a single mosquito from a population of susceptible, 25-fold, and 125,000-fold resistant mosquito larvae. The sequencing results revealed 3 groups of α-glucosidase genes; (1) normal, (2) Asp95 → Ala, Ala113 → Ser, Thr178 → Gln, Thr430 → Ser, Pro450 → Ser, H466 → Tyr, Gly541 → Ser, and Met556 → Thr, (3) deletion at nucleotide 475 that leading to a frameshift and premature protein translation. The mutant gene was found in 10% the susceptible population. However, in the 100,000-fold resistant population, the mutant genes were found in all mosquito larvae.

B3_B0075 Cloning and identification of cadherin-like protein, a putative *Bacillus thuringiensis* toxin receptor, in *Aedes aegypti* mosquito larva.

Mongkhon Panyot and Kusol Pootanakit
Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakhorn-Pathom 73170.
E-mail address: g4737246@student.mahidol.ac.th

Abstract: The insecticidal crystal proteins (Cry toxins), produced from bacterium *Bacillus thuringiensis*, bind to specific receptors on the midgut epithelial cells of susceptible insects, leading to pore formation and death of the insects. Cadherin-like protein is considered one of the receptors to Cry toxins in many insect species; but its expression in the gut of larvae Ae. aegypti has not yet been identified. In this study, using RT-PCR, we have identified and cloned cadherin-like protein from the midgut of 2-day old *Ae. aegypti* larvae. So far, we have obtained 1,498 nucleotides, encoding 498 amino acid located at the 3' end of the gene. The obtained partial sequence has 57% amino acids sequence similarity to *An. gambiae* cadherin-like protein.

B3_B0079 PARTIAL CLONING OF 5-AMINOIMIDAZOLE RIBONUCLEOTIDE (AIR) CARBOXYLASE GENE FROM *KLUYVEROMYCES MARXIANUS*.

Wanatchara Impath, Panomwan Wangspa and Rungrach Wangspa
Economic Plant Genome Research and Service Center, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand.
Email: rungrach@chiangmai.ac.th, Fax: +66-5389-2259

Abstract: The aim of this study is to partially identify the nucleotide sequence of the AIR carboxylase gene, *ADE2*, from the yeast *Kluyveromyces marxianus*. PCR primers were designed from conserved sequences recognized by analysis of yeast ADE2 nucleotide sequences available in Genbank. PCR amplification using an annealing temperature of 57°C produced a single 560-bp band from *K. marxianus* genomic DNA template. The PCR product was extracted, ligated into the vector pTZ57R/T. EcoRI-HindIII double digestion was used to confirm the presence of the insert in the recombinant plasmid. The nucleotide sequence of the insert was found to be most similar to ADE2 from *K. lactis* (more than 80% identical). The deduced amino acid sequence contained two AIR carboxylase conserved domains, PurK, at the N-terminal, and AIRC, at the C-terminal. The results confirmed that the insert was amplified from *ADE2* of *K. marxianus*.

B3_B0086 GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE BLUE SWIMMING CRAB *Portunus pelagicus* DETERMINED BY SSCP ANALYSIS

Natechanok Thamniemdee[1], Bavornlak Khamnamtong[2,3], Sirawut Klinbunga[2,3] and Piamsak Menasveta[3,4]

[1] Program of Biotechnology, [2]Center of Excellence for Marine Biotechnology, [4]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[3] National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120. e-mail: sirawut@biotec.or.th

Abstract: Genetic diversity and population differentiation of the blue swimming crab (*Portunus pelagicus*) was examined by SSCP analysis. Primers originally designed from MRRAP38 and BSCAFLP-454 were tested against genomic DNA of *P. pelagicus*. The positive amplification product of each gene was cloned and sequenced. Sequence-specific primers (BSCSCAR1-F/R and BSCSCAR2-F/R) were designed and tested against *P. pelagicus* from Chanthaburi, Surattani, Ranong and Krabi (N = 111). In total, 19 and 7 polymorphic SSCP fragments were found and generated 61 and 23 SSCP genotypes,

respectively. Genetic distance between pairs of geographic samples was 0.0043 – 0.0235. Significant genetic heterogeneity was found across overall investigated samples ($P < 0.0001$) suggested that the gene pool of *P. pelagicus* in Thai waters is not panmictic but fragmented into several populations. Pairwise genetic differentiation analysis revealed a fine scale genetic subdivision (i.e. between geographic samples within and between coastal regions) in this species ($P < 0.05$). In addition, a species-diagnostic marker for authentication of *P. pelagicus* was developed. The COI gene segment (706 bp) amplified from universal primers was cloned and sequenced. A new pair of primers (PP-COI_{270}-F/R) was designed and tested for species specificity. The expected product (270 bp) was specifically found in *P. pelagicus* ($N = 111$) but not in mud crabs (*Scylla serrata*, $N = 7$, *S. oceanica*, $N = 18$ and *S. tranquebarica*, $N = 10$) and *Charybdis cruciferа* ($N = 6$).

B3_B0115 CELL SURFACE DISPLAY OF ORGANOPHOSPHORUS HYDROLASE USING CYANOBACTERIAL SomA SYSTEM

Chutirat Asawathep, Sirirat Fa-aroonsawat and Wipa Chungjatupornchai
Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand.
E-mail: mimpku60@yahoo.com

Abstract: It has been reported that the activated cyanobacteria-reactor is an economical method for treating wastewater; organophosphorus hydrolase (OPH) encoded by *opd* gene of *Flavobacterium* sp. can effectively detoxify organophosphorus compounds which are highly toxic and used extensively as agricultural and domestic pesticides. Therefore, recombinant cyanobacteria with surface expressed OPH could be used as a novel method for biodegradation of pesticides. In this study, the *Synechococcus* outer membrane (SomA) protein was used as an anchoring motif to surface display the OPH on cyanobacterium *Synechococcus* PCC7942. The *somA-opd* gene fusion under the control of *somA* promoter (P_{somA}) and tRNA promoter (P_{tRNA}) of *Synechococcus* were constructed in a shuttle vector and transformed into *Synechococcus*. Proteinase accessibility assay showed that OPH were surface expressed on Synechococcus harboring P_{somA}-*somA-opd* and P_{tRNA}-*somA-opd*, however, the OPH activity of the gene under P_{somA}

B3_B0120 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FOR IDENTIFYING THAI AND MYANMAR STRAINS OF *WUCHERERIA BANCROFTI*: IMPLICATIONS FOR THE CONTROL PROGRAM IN THAILAND

Alisa Junpee[1], Surang Nuchprayoon[1,*], Yong Poovorawan[2]
[1] Lymphatic Filariasis Research Unit, Department of Parasitology, [2]Department of Pediatrics, and Chula Medical Research Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[*] Corresponding author

Abstract: Lymphatic filariasis mainly caused by filarial nematodes, *Wuchereria bancrofti* and *Brugia malayi*, is still a major public health problem. We study, microfilarial periodicity, morphometry, random amplified polymorphic DNA (RAPD), and phylogenetic dendrogram to differentiate the Thai *W. bancrofti* (nocturnal subperiodicity) and the Myanmar (nocturnal periodicity) strains. The body length, cephalic space length, and cephalic space width of the Thai strain were significantly larger than those of the Myanmar strain. However, overlapping mean values of those parameters made it not practical for field application. By using RAPD primer 5'-dGGTGCGGGAA-3', the number of DNA fragments amplified from each sample ranged from 5-11. The specific 300 bp band was specific for the Myanmar *W. bancrofti* strain. The phylogenetic dendrogram indicated two genetically distinct clusters of the Thai and Myanmar strains. This is the first report on the genetic polymorphism of the Thai *W. bancrofti* and the Myanmar strains. The RAPD marker is useful for control and elimination program of lymphatic filariasis in Thailand.

B3_B0132 TEMPORAL GENETIC VARIATION OF JUVENILE ORANGE-SPOTTED GROUPER *Epinephelus coioides* (Hamilton, 1822) COMMERCIALLY COLLECTED FROM KANGTANG DISTRICT, TRANG

Panuwat Pumitinsee[1] Wansuk Senanan[1] and Uthairat Na-Nakorn[2]
[1] Department of Aquatic Science, Faculty of Science , Burapha University, Chonburi 20131, Thailand
[2] Department of Aquaculture, Faculty of Fisheries , Kasetsart University, Bangkok 10900, Thailand

ABSTRACT: In a fishing ground, fingerlings of orange-spotted grouper (*Epinephelus coioides*) are consistently exploited year round. A question pertaining to fisheries management is whether the fingerlings collected at different time in a year come from one reproductively isolated population. We analyzed temporal genetic variation of grouper fingerlings collected from Trang province using five polymorphic microsatellite loci (6-21 allele/locus, average observed heterozygosity = 0.12-1). Genotypic frequencies of microsatellite loci across most groups did not deviate from those expected under the Hardy-Weinberg equilibrium. Cluster analyses of genetic distances among fingerlings collected in different months indicated that fingerlings collected in July may be genetically distinct from ones collected in other sampling periods (Cavalli-Sforza and Edwards Genetic distance = 0.03-0.05; Exact test, $p<0.001$). This genetic differentiation may reflect family or sub-population differences. The combination of genetic and other biological data may aid management of this grouper population in this important fishing area.

B3_B0135 PATERNITY ANALYSIS OF GREEN TURTLE (*Chelonia mydas*) HATCHLINGS USING MICROSATELLITE MARKERS

Chaowalee Jaisuk[1] , Wansuk Senanan[1] and Somchai Manamunsap[2]
[1] Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi, 20131, Thailand
[2] Sea turle Conservation Station , Department of Marine and Coastal resources, Mannai Island, Rayong, 21190, Thailand

ABSTRACT: Conservation and rehabitation of endangered species, such as sea turtles, require ecological knowledge. We used microsatellite DNA fingerprinting (3 - 5 loci ; cm72, cm3, cm58, cc117 ; Fitzsimmons et al., 1995 and cc7 ; Fitzsimmons, 1998) to evaluate the proportions of males and females green turtle (*Chelonia mydas*) contributing to hatchlings at Kham Island, Chonburi. We analyzed four females and their hatchlings (two clutches/female and 10 - 20 hatchlings/clutch) collected during a nesting season April – September 2004. Preliminary results indicated that each female mated with at least 2 – 4 males. The results may suggest that multiple paternity is quite common in this population (100% of the females examined). This information may help the planning of conservation efforts and a breeding program that is consistent with the natural mating behavior.

B3_B0136 EXPRESSION AND BIOCHEMICAL ASSAY OF ARGONAUTE PROTEIN FOR THE ROLE IN RNA INTERFERENCE OF BLACK TIGER SHRIMP (*Penaeus monodon*)

Narakorn Khunweeraphong Sakol Panyim and Apinunt Udomkit
Institute of the Molecular Biology and Genetics, Salaya Campus, Mahidol University, Nakhon Pathom 73170, Thailand;
e-mail address: narakorntom@gmail.com

Abstract: Argonaute protein is the core component of RISC that plays critical role in sequence-specific mRNA cleavage in RNA interference (RNAi) gene silencing pathway which is responsible for antiviral mechanism in eukaryotes. The understanding of RNAi in shrimp is therefore essential for development of effective viral defense strategy in the future. Argonaute protein contains of 2 conserved regions, PAZ and PIWI domains. The recombinant Argonaute and recombinant PAZ domain were expressed from pET-15b vector as N-terminal hexahistidine fusion-tagged proteins in *Escheria coli* (BL21(DE3)pLysS). The soluble proteins were further purified by Ni^{2+} column chromatography. The RNA binding domain function of Argonaute and the PAZ domain was investigated by Electrophoretic Mobility Shift Assay (EMSA) with ^{32}P-labeled ssRNA. By using ssRNA, dsRNA or ssDNA as competitors, the result revealed that the PAZ domain bind preferentially to RNA either single- or double-stranded, than to DNA. The function of Argonaute protein in sequence-specific mRNA cleavage will be further characterized by mRNA cleavage assay.

B3_B0163 Contribution of active site residues to key enzyme-substrate interaction of the dengue virus NS3 serine protease

Wanisa Salaemae and Asst. Prof. Gerd Katzenmeier
Institute of the Molecular Biology and Genetics, Mahidol University Salaya Campus, Puttamonthol Nakornpathom, Thailand
E-mail:wanisa_s@hotmail.com

Abstract: The NS3 protease of dengue virus serotype 2 represents an attractive target for the development of antiviral inhibitors. Although the three-dimensional structure and active sites of the NS3 protease domain have been determined, the mechanism of substrate recognition is characterized only to a limited extent. To elucidate enzyme-substrate interactions, we targeted nine residues at the S1 and S2 pockets in the active site of dengue virus protease by site-specific mutagenesis. Residues Leu115, Asp129, Gly133, Thr134, Tyr150, Gly151, Ser163, and Ile165 at S1 and Asn152 at S2 were replaced with alanine. From SDS-PAGE analysis of autoproteolytic cleavage at the NS2B/NS3 junction, compared to the wild type, the L115A, D129A, G133A, T134A, N152A, S163A and I165A mutants demonstrated inefficient autoprocessing at several levels, whereas in the Y150A and G151A mutants enzyme activity appeared to be almost completely abolished. From enzymatic activity assay with the fluorogenic peptide substrate GRR-AMC, only L115A mutant has slightly higher catalytic efficiency than wild type whereas the others present lower activity. Similar to autoprocessing, Y150A and G151A substitutions inactivate the enzyme.

B4_B0008 EFFECTS OF AQUEOUS EXTRACTS FROM SIAMESE'S ROOT (*CASSIA SIAMEA* BRITT.) ON THE DEATH AND THE TEGUMENTAL SURFACE CHANGING OF THE TREMATODE, *STELLANTCHASMUS FALCATUS* USING SCANNING ELECTRON MICROSCOPY

Chalobol Wongsawad [1], Chaiyaporn Rukrowm [1], Pheravut Wongsawad [1], Kanda Whangchai [1]
[1] Department of Biology, Faculty of Science, Chiang Mai University 50202
E-mail: cwongsawd@yahoo.com

Abstract: The appropriated concentration and time of aqueous extracts from Siamese cassia's root (*Cassia siamea* Britt.) which effect on trematode were investigated on *in vitro* effect on movement and tegumental surface of trematode (*Stellantchasmus falcatus*) using light microscope and the SEM (Scanning Electron Microscopy) observation of the death trematode was examined the tegumental surface of the body. The worms treated with aqueous root extract at concentration 6.25%, 12.5%, 25% and 50% in Tyrode' s solution at 1, 6, 12 and 24 hours were examined the death using Tyrode's solution as a control. The result showed that extract of 6.25%, 12.5% and 25% can kill all the worms within 6 hours, which 50% extract concentration can kill worms 92.5 % within 1 hour. The examination found that the trematode in the extract of 6.25% solution showed loss of the scales at 6 hours and curving at the edge of scales at 12 hours, which the trematode in the extract of 12.5% solution shown loss of the scales and curving at the edge of scales at 6 hours. Whereas the trematode in the extract of 25% and 50% solution found the same changing of the tegument as the trematode in the extract of 12.5% but more effectively as loss of the scale at 1 hour, found the rupturing and the tegument tear off from a tegumental surface at 12 hours.

B4_B0009 PRODUCTION OF 17α-HYDROXYPROGESTERONE FROM PROGESTERONE BY BIOTRANSFORMATION

Jiradej Manosroi[1,2*], Suda Saowakhon[2] and Aranya Manosroi[1,2]
[1] Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand 50200.
[2] Natural Product Research and Development Center, Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai, Thailand 50200.
E-mail address: pmpti006@chiangmai.ac.th, saowakon_s@hotmail.com, and pmpti005@chiangmai.ac.th

Abstract: The objective of this study was to investigate the production of 17α-hydroxyprogesterone (17α-OHP) from progesterone by hydroxylation reaction using biotechnological process. Nine strains of fungi were investigated. They were cultured in enriched medium containing 0.3 mg/ml of progesterone and shaken at 200 rpm, 25±2°C. The qualitative and quantitative analysis of 17α-OHP were TLC and HPLC. The results showed that C. *lunata* ATCC 12017 gave the maximum yield of 55.00±4.25% at 24 hours, whereas C. *blakesleeana* ATCC 8688a gave the yield of 35.25±4.86% at 48 hours. The other strains gave only trace of ADD in the range of 1.46%-6.10% The result from this study can be used as an alternate for the currently used chemical method for the production of 17α-OHP and other substances which need 17α-hydroxylation reaction.

B4_B0026 ISOLATION OF BIOSURFACTANT PRODUCED BY *EXOPHIALA DERMATITIDIS*

Paramaporn Chiewpattanakul and Benjamas Thanomsub
Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand E-mail:paramaporn_chiew@yahoo.com, Fax: (662)321-2437

Abstract: Biosurfactants are amphiphatic compounds produced by microorganisms which have a number of advantages over the chemically synthesized because of their structure diversity, biodegradable, wide range of temperatures, pH and salinity stability. These properties render wide applications of biosurfactants such as bioremediation, pollution control, textiles, cosmetic and medicine. In this study, we isolated the microorganisms produced biosurfactant from oil-contaminated soil in Thailand. The total broth of the isolated microorganisms was tested for biosurfactant production using a drop collapse test. One fungus isolated from soil waste in palm oil factory, Songkhla province demonstrated high surfactant activity. The microscopic study showed yeast-like cells, some septate hyphae with annelides and black colony on YM agar. The 28s rRNA sequence showed high similarity to *Exophiala dermatitidis*. The emulsification stability of the biosurfactant produced from this strain was stable up to 48 hours. This strain was able to grow in 40°C and showed high production when using soy bean or palm cooking oil as substrate. These findings implied the application and economic impact for using this fungus in biosurfactant production at relative high temperature and through the use of inexpensive substrate which is much available in Thailand.

B4-B0030 Prediction of Tertiary Structure of Anthocyanidin Synthase (ANS) from *Camellia sinensis*

Siam Popluechai[a], Lalida Vachangken[a], Chinae Thumarongthum[b], Prapassorn Damrongkool[a] and Ekachai Chukeatirote[a]
[a] School of Science, Mae Fah Luang University, Chiang Rai 57100 Thailand
[b] National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani 12120 Thailand

Abstract: In this paper, the structure of anthocyanidin synthase (ANS) from *Camellia sinensis* was elucidated using homology modeling. The models were generated based on the ANS structural template of *Arabidopsis thaliana* and subsequently assessed in terms of their geometry and Ramachandran diagrams. Our results suggest that the model constructed is possible and thus can be used for further study related to enzymatic structure, function and kinetics.

B4_B0038 CLONING OF A CARCININ-LIKE, SINGLE WHEY-ACIDIC-DOMAIN, ANTIMICROBIAL PEPTIDE FROM THE HEMOCYTES OF THE MUD CRAB, *SCYLLA PARAMAMOSAIN*

Chanprapa Imjongjirak[1], Piti Amparyup[2,3], Anchalee Tassanakajon[3] and Siriporn Sittipraneed[4]
[1] Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand,
[2] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong1, Klong Luang, Pathumthani, 12120, Thailand
[3] Shrimp Molecular Biology and Genomics Laboratory, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[4] Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

Abstract: Carcinin-like antimicrobial peptide, a whey acidic protein (WAP) domain-containing antimicrobial peptide, was cloned from the circulating hemocytes of the mud crab, *Scylla paramamosain* by PCR technique. Nucleotide sequencing analysis revealed the existence of 7 putative isoforms of carcinin-like antimicrobial peptide in *S. paramamosain* (CarSp1-CarSp7). The most commonly expressed transcript of the protein was CarSp1. These variants contained an open reading frame (ORF) of 336 bp encoding for 111 amino acid residues with predicted molecular weight of approximately 10 kDa. The inferred amino acid sequence of this peptide was found to be similar to carcinin antimicrobial peptide isolated from the shore crab, *Carcinus maenas*. Phylogenetic analysis of this new peptide shows that it is most related to other antimicrobial crustin peptides. RT-PCR was used to analyze the expression of the CarSp1 in *S. paramamosain* tissues. The result showed that CarSp1 was expressed in hemocytes, gill, intestine, muscle but not in hepatopancreas and eyestalk, respectively.

B4_B0040 IDENTIFICATION OF IRRADIATED MUNG BEANS USING DNA COMET ASSAY

Wanwisa Sudprasert, Pannee Pakkong, and Sirikan Nawapan
Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Phahonyothin Road, Bangkok, Thailand.
E-mail: fsciwasu@ku.ac.th, Tel: (662)942-8481, Fax: (662)579-5530

Abstract: A study was carried out to obtain the most appropriate condition for identification of irradiated mung beans by DNA comet assay. The study emphasized on the two main parameters, amount of sample used and lysis time. The amount of 300 and 450 mg mung beans were irradiated by Cs-137-gamma radiation to obtain radiation dose of 0, 0.5 and 1 kGy. The cells from beans were extracted with cold phosphate buffered saline (PBS). The extracted cells were mixed with 0.8% low-melting agarose before embedded on a microscope slide precoated with 0.5% agarose. Then the cell wall and nucleus was digested with 2.5% sodium dodecyl sulphate in tris-borate EDTA (TBE) for 25 and 30 minutes. DNA fragments were separated by gel electrophoresis at 2 volts/cm for 2 minutes using TBE buffer as an electrolyte. After silver staining, the slide was evaluated through an ordinary transmission microscope. It was found that condition when using 450 mg mung beans and 30 minutes lysis time gave the best result. The fragmented DNA stretched toward the anode and had the shape like a comet. The length of the comet tail increased with increasing doses of gamma rays. For unirradiated mung beans (control), most of cells were round shape or short tails. It can be concluded that 450 mg sample and lysis time of 30 minutes was the optimum condition for identification of irradiated mung beans by means of DNA comet assay.

B4_B0045 Preparation and Characterization of Inclusion Complexes Containing Fixolide, a Synthetic Musk Fragrance and Cyclodextrins.

PUTCHARIN CHITTITEERANON[1], SUGANYA SOONTAROS[2], and PIAMSOOK PONGSAWASDI[2]
[1] M.Sc.Program in Biotechnology
[2] Starch and Cyclodextrin Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

Abstract: AHTN (7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene), commercially known as fixolide or tonalide, is a popular synthetic fragrance material added in personal care and household products. AHTN is degraded under light exposure, high temperature and is poorly soluble in water. The aim of this study was to increase the stability and dissolution rate of AHTN by inclusion complex formation with cyclodextrins. Solid complexes of AHTN with β-cyclodextrin, methyl(MβCD), and hydroxypropyl (HPβCD) derivatives were prepared by physical mixing, co-precipitation, kneading, and freeze-drying methods with three molar ratios (1:1, 1:2, and 1:3). DSC and FTIR were used to confirm the inclusion complex. The results obtained from DSC and FTIR indicated that AHTN effectively formed inclusion complex with βCD and MβCD at the mole ratio of 1:2 by co-precipitation and kneading method, respectively. AHTN in the complex from attained higher thermal and UV stability, higher and faster dissolution rate than its free form.

B4_B0047 DISCOVERY OF *CYCLIN A*, A GENE REGULATION CELL CYCLES IN OVARIES OF THE GIANT TIGER SHRIMP *PENAEUS MONODON*

Virak Visudtiphole[1] and Sirawut Klinbunga[1,2]
[1] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani 12120
[2] Center of Excellence for Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330

Abstract: Cyclin A is a regulatory subunit of cyclin dependent kinase (Cdk) 1 and 2 to control eukaryotic cell division cycles. The partial transcripts of *cyclin A* (648 bp) of the giant tiger shrimp (*Penaeus monodon*) were identified through an EST approach. The full length cDNA of *cyclin A* was identified by RACE-PCR. Two *cyclin A* variants that share identical open-reading frame (ORF) but 3' UTR sequence polymorphism were found. The full transcript of *cyclin A* in *P. monodon* is 2092 bp in length and ORF is 1,326 bp, encoding a putative protein of 441 amino acid residues with the 5' and 3' UTR of 119 and 650 bp, respectively. The cyclin box domain typically found in this protein family was predicted and located at positions 217^{th} – 312^{th}. RT-PCR was carried out to determine expression patterns of this transcript in ovaries and testes of juvenile and broodstock *P. monodon*. The expression levels of *cyclin A* in ovaries was significantly greater than that in testes of both juveniles (N = 4) and broodstock (N = 8) P. monodon ($P < 0.05$). Nevertheless, *cyclin A* displayed comparable expression levels in undeveloped (gonadosomatic index, GSI = 0.65, 1.10 and 1.43) and developed (GSI = 5.69) ovaries of *P. monodon* broodstock.

B4_B0049 IDENTIFICATION OF GENES EXHIBITING SEX-SPECIFIC AND/OR DIFFERENTIAL EXPRESSION PATTERNS IN OVARIES OF THE GIANT TIGER SHRIMP *Penaeus monodon*

Kanchana Sittikankeaw[1], Sirawut Klinbunga[2,3] and Piamsak Menasveta[3,4]
[1] Programarine of Biotechnology, [2]Center of Excellence for Marine Biotechnology, [4]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[3] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klongluang, Pathumthani 12120

Abstract: Expression patterns of 162 gene homologues in ovaries (*N* = 6) and testes (*N* = 6) of *P. monodon* were examined

by RT-PCR. Fourteen gene homologues were specifically expressed in ovaries and 58 gene homologues revealed differential expression in ovaries of broodstock-sized *P. monodon* ($P < 0.05$). RACE-PCR of *Asparaginyl tRNA synthetase (PMAtNS)* was carried out and the full length cDNA of this gene was 1857 bp (ORF = 1680 bp encoding a protein of 560 amino acids). The RT-PCR product of 22 gene homologues was analyzed by SSCP. Patterns of *PMAtNS* of female shrimps with low (GSI < 1.5%) and moderate GSI values were different. Additional expressed gene products were observed in developed ovaries (GSI = 1.89, 2.02 and 2.13). This suggested that *PMAtNS* may play an important role during ovarian development of *P. monodon*.

B4_B0053 Effect of sodium chloride on proline profiles and leaf growth characteristics in indica rice (*Oryza sativa L. ssp indica*) varieties

Wasinee Pongprayoon[1], Aussanee Pichakum[1], Sittiruk Roytakul[2], Suriyan Cha-um[2] and Chalermpol Kirdmanee[2]
[1] Department of Plant Science, Faculty of Science, Mahidol University, Rama VI Rd, Payathai, Bangkok 10400,Thailand.
[2] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Rd, Klong Luang, Pathumthani 12120, Thailand.

Abstract: Salinity is one of the most serious problems affecting on agricultural productivity worldwide, especially rice crop. The osmoregulation system through proline accumulation has been reported as an effective defense mechanism. The aim of this research was to study the proline accumulation in salt tolerant and salt sensitive rice varieties at various NaCl concentrations. Eight varieties of salt-tolerant rice varieties, such as Pokkali, Hawm Pae-palo, Hawm Sa-dung, Hawn Jun, Hawm Nang Nuan, Hawm Tang, Hawm Mae Jun, Hawm Phrae and a salt-sensitivity (IR29) grown *in vitro* under photoautotrophic condition were treated with 0, 171, 342, 513, and 684 mM NaCl for 4 days. Proline contents in all rice varieties were increasingly accumulated along with the increase in salt concentration. The proline content of most varieties was similar except in Hawm Pae-palo showed a dramatically increase. In addition, the salt-tolerant varieties significantly showed the higher percentage of green leaf area than the salt-sensitive variety.

B4_B0058 DNA Fingerprinting of *Phyllanthus* species by RAPD Genetic Markers

Juthatip Manissorn, Suchada Sukrong, Nijsiri Ruangrungsi
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

Abstract: Ramdom amplified polymorphic DNA (RAPD) markers were used to identify and construct the genetic relationships in *Phyllanthus* species (Euphorbiaceae). *Phyllanthus species* have been uses as medicinal plants, edible fruits, and ornamental plants. Twelve *Phyllanthus* species, *P. amarus, P. urinaria, P. emblica, P. taxodiifolius, P. collinsae, P. acidus, P. pulcher, P. debilis, P. virgatus, P. acutissimus, P. polyphyllus, P. reticulatus*, and two unknown species Phyllanthus sp. 1, and Phyllanthus sp.2 were screened for the genetic analysis by thirty deca-oligonucleotide primers. From nine primers, OPS-01, OPS-03, OPS-07, OPS-08, OPS-12, OPS-19, SD-2, SD-4 and SD-7, DNA fingerprints which revealed polymorphism were generated for identification *Phyllanthus* species. RAPD fingerprint can identify all of *Phyllanthus* species. RAPD bands were scored and grouped by distance analysis using a pair-wise genetic similarity according to the index of Nei and Li. Dendrogram was generated by the Neighbor-Joining (NJ) cluster analyses. Four clusters were revealed: group I (*P. virgatus, P. polyphyllus, Phyllanthus* sp.1, *P. acutissimus*) group II (*P. pulcher, P. amarus, P. taxodiifolius, P. reticulatus*) group III (*P. urinaria, P. emblica, P. collinsae, P. acidus*) and group IV (*Phyllanthus* sp.2, *P. debilis*). Variation in DNA fingerprint of the *Phyllanthus* species indicates the efficiency of RAPD genetic marker for the identification and construction of genetic relationship.

B4_B0066 MONOCLONAL ANTIBODIES AGAINST RECOMBINANT VP3 PROTEIN OF TAURA SYNDROME VIRUS

Piangjan Poyoi[1], Parin Chalvisuthangkura[1], Thanawan Tejangkura[1], Siwaporn Longyant[1], Sombat Rukpratanporn[2], Weerawan Sithigorngul[1], and Paisarn Sithigorngul[1]
[1] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok10110, Thailand.
[2] Center of Excellence for Marine Biotechnology at Chulalongkorn University, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok 10330, Thailand.

Abstract: Monoclonal antibodies (MAbs) specific to VP3 structural protein of Taura syndrome virus (TSV) were generated from a mouse immunized with recombinant GST-VP3 fusion protein expressed in E. coli. The hybridoma clones were selected by dot blotting, Western blotting and immunohistochemistry. Four MAbs specific to VP3 and one MAb specific to GST were selected and cloned to establish the permanent cell lines. All MAbs specific to VP3 were able to detect TSV infection in shrimp by Western blotting and immunohistochemistry without cross reactivity to shrimp tissues. Further development for simple and convenient strip test is in progress.

B4_B0067 HIGH EXPRESSION OF *BACILLUS THURINGIENSIS CYT1AA1, CYT2AA2* GENES AND *BACILLUS SPHAERICUS* BINARY TOXIN GENE IN *BACILLUS THURINGIENSIS* SUBSP. *DARMSTADIENSIS* STRAIN 495 RESULTS IN HIGH TOXICITY TOWARD CULEX *QUINQUEFASCIATUS* LARVAE

Ritthironk Sae-lee[1], Monrudee Ratlerdkarn[2], Boonhiang Promdonkoy[2], Usavadee Thavara[3], and Mongkon Audtho[2]
[1] Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand.
[2] National Center for Genetic Engineering and Biotechnology, NSTDA, 113 Phaholyothin Rd., Klong Luang, Pathumthani, Thailand. E-mail: mongkon@biotec.or.th
[3] Division of Medical Entomology, Department of Medical Sciences, NIH, Ministry of Public Health, Nonthaburi, Thailand.

Abstract: *Bacillus thuringiensis* subsp. *darmstadiensis* strain 495 is not toxic against *Culex quinquefasciatus* larvae, while *B. darmstadiensis* strain 73E10 produces a low amount of Cyt2Aa2 protein and is not effective against the larvae. In this study, *cyt1Aa1* gene from *B. thuringiensis* subsp. *israelensis*, *cyt2Aa2* gene from *B. darmstadiensis* strain 73E10, and binary toxin gene from *B. sphaericus* strain 2362 was cloned by using specific primers in PCR reaction, and inserted into shuttle vectors, pBCX, and pRB373. The DNA constructs were used to transform *B. darmstadiensis* strain 495 by electroporation. High expression of each protein was found in the bacteria harboring each construct and the bacteria were highly toxic against the mosquito larvae. LC_{50} of the bacteria producing Cyt1Aa1, Cyt2Aa2, and binary toxin, were 8.9×10^3, 8.4×10^2, and 3.2×10^2 cells/ml, respectively.

B4_B0074 A preliminary study on transformation of *Dendrobium primulinum* Lindl. with antisense ACC oxidase

Kuntida Duangsee[1] and Sumontip Bunnag[2]

[1] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand 4002; e-mail: kun0155@yahoo.com

[2] Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand 4002

Abstract: The objectives of this research were to establish transformation procedure for *Dendrobium primulinum* Lindl. by using *Agrobacterium tumefaciens* LBA4404 (pCAMBIA1305.1). Efforts were directed at the following: selecting the suitable concentration of cefotaxime for elimination *Agrobacterium* and selecting the suitable concentration of hygromycin for selection of transgenic *D. primulinum*. The results showed that the highest concentration of cefotaxime that protocorms of *D. primulinum* could tolerate were 150 mg/l. The protocorms of *D. primulinum* were completely inhibited by hygromycin concentration at 15 mg/l. The antisense ACC oxidase were constructed in plasmid pCAMBIA 1305.1 which containing gus gene as reporter gene and hpt gene as selectable marker gene. The positive results of GUS assay revealed the GUS activity. The percentage of GUS activity was 60% and the optimal co-cultivation time was 60 minutes.

B4_B0084 EXPRESSED SEQUENCE TAG ANALYSIS FOR ISOLATION AND CHARACTERIZATION OF SEX DIFFERENTIATION-RELATED GENES IN THE GIANT TIGER SHRIMP (*Penaeus monodon*)

Bachanimuk Preechaphol[1], Rungnapa Leelatanawit[1], Kanchana Sittikankaew[1], Sirawut Klinbunga[2,3]* and Piamsak Menasveta[2,4]

[1] Program of Biotechnology, [2]Center of Excellence for Marine Biotechnology, Faculty of Science, Chulalongkorn University, Bamgkok 10330

[3] National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120. E-mail: sirawut@biotec.or.th

Abstract: Sex-related genes expressed in vitellogenic ovaries of the giant tiger shrimp (*Penaeus monodon*) were identified by an EST approach. A total of 1051 clones were unidirectional sequenced from the 5' terminus. Nucleotide sequences of 758 ESTs (71.7%) significantly matched known genes previously deposited in the GenBank (E-value < 10^{-4}) whereas 308 ESTs (29.3%) were regarded as newly unidentified transcripts (E-value > 10^{-4}). Five hundred and fifty-nine putative transcripts (87 contigs and 472 singletons) were obtained. *Thrombospondin* (TSP; 79 clones, 7.5% of clones sequenced) and *peritrophin* (87 clones, 8.3%) predominated among characterized transcripts. Several full length transcripts were also isolated. A gene homologue encoding chromobox protein (PMCBX, ORF of 567 nucleotides encoding a protein of 188 amino acids) which is recognized as a new member of the HP1 family was identified. Expression patterns of 14 of 25 sex-related gene homologues in ovaries and testes of *P. monodon* were examined by RT-PCR. *Female sterile* and *ovarian lipoprotein receptor* displayed female-specific expression whereas almost all of the investigated transcripts (*e.g. phosphatidylinositol 4 kinase; nuclear autoantigenic sperm protein, NASP; small androgen receptor-interacting protein, SARIP* etc.) showed differential expression patterns in ovaries of *P. monodon* broodstock. A homologue of *ubiquitin specific preteinase 9, X charomosome (USP9X)* revealed differential expression between ovaries and testes of broodstock-sized *P. monodon* (N = 13 and 11, $P < 0.05$) but temporal female-specific expression in 4-month-old shrimps (N = 5 for each sex).

B4_B0087 DNA FINGERPRINTS AND MOLECULAR DIVERSITY OF FOUR SPECIES OF *PHYLLANTHUS* ASSESSED THROUGH RAPD ANALYSIS

Nantawan Kanawapee, Piyada Theerakulpisut, Sumontip Bunnag, Duangkamol Maensiri and Pranom Chantaranothai

Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

Abstract: Four species of *Phyllanthus* (*Phyllanthus amarus* Schum. & Thonn., *P. debilis* Klein ex Willd., *P. urinaria* L. and *P. emblica* L.) were collected from 7-8 locations mainly in northeastern Thailand. *P. amarus*, *P. urinaria* and *P. debilis* are small herbaceous plants, morphologically very similar and commonly occur as overlapping populations in the same location. They also have similar vernacular names of Luk-tai-bai and Ya-tai-bai. The high level of similarity can lead to collection of wrong plant species for medicinal uses. Randomly amplified polymorphic DNA is therefore adopted to assist in species identification especially for species which are used as medicinal herbs. Twenty decamer primers were used to amplify DNA from each of the three *Phyllanthus* species collected from different locations. Amplification of different plant species using the same primer resulted in distinctive banding patterns characteristic of each species and can be used for species identification. RAPD fingerprints of the same plant species collected from different locations are generally very similar. The UPGMA analysis showed similarity coefficients of 0.67, 0.56 and 0.60 among different populations of *P. amarus*, *P. urinaria* and *P. debilis* respectively. Cluster analysis based on data from RAPD banding pattern using eight primers to amplify samples of the three

herbaceous *Phyllanthus* species and one tree species (*P. emblica* L.) clearly separate different plant species into different groups, with *P. urinaria, P. debilis* and *P. emblica* more closely related to each other than to *P. amarus*.

B4_B0090 BATCH ALCOHOL PRODUCTION FROM SLOP

Yupakanit 2 Puangwerakul
Faculty of Biotechnology, Rangsit University, Patumthani, Thailand
E-mail: lombiotec@yahoo.com, Fax: (662) 997-2220 ext.3661

Abstract: Comparison of difference batch fermentation process, batch process, step wise fed batch process and exponential fed batch process were assigned. Alcohol production from molasses mixed with slops at the ratio 1:1 in 1,500 liter fermenter was prepared 16% (W/V) invert sugar content in medium using *Saccharomyces cerevisiae* RSU no.10 $1.11x10^8$ cells/ml in 10% inoculum size for starter was studied. The result showed that 8.5 % alcohol (V/V) was received in 12 hours of fermentation period by the process of step wise fed batch and exponential fed batch was faster than conventional batch process. It was found that the relationship between desired alcohol content and the highest number of lived yeast cells was at 10^9 cells/ml.

B4_B0094 SODIUM CHLORIDE SALT AFFECTED ON PHOTOSYNTHETIC EFFICACY IN SALT-TOLERANT AND SALT-SENSITIVE RICE (Oryza sativa L. ssp Indica) VARIETIES.

Thanaphol Boriboonkaset[1], Aussanee Pichakum[1], Sittiruk Roytakul[2], Suriyan Cha-um[2] and Chalermpol Kirdmanee[2]
[1] Department of Plant Science, Faculty of Science, Mahidol University, Rama VI Rd, Payathai, Bangkok 10400
E-mail: iamthanaphol@yahoo.com, Fax: (662)354-7172
[2] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Rd, Klong Luang, Pathumthani 12120

Abstract: Soil salinity is an abiotic stress, which directly limited on crop productivity, especially in rice production. In previous studies, photosynthesis alteration by salinity was a main restriction rice crop yield reduction. The objective of this investigation was to evaluate the water oxidation in salt tolerant and salt sensitive rice seedlings with or without root system grown under 342 mM NaCl salt stress. Osmolarity, pigment content, maximum quantum yield (Fv/Fm), photon yield of PSII (Φ_{PSII}) and non-photochemical quenching (NPQ) parameters of salt-tolerant varieties; Pokkali and Homjan (HJ), and salt-sensitive varieties; IR29 and Pathumthani 1 (PT1) were evaluated. The results showed that osmolarity of salt-stressed leaves in both groups were continuously increased with increasing the NaCl exposure times. The chlorophyll degradation in salt-stressed rice was directly reduced on water oxidation and electron transportation in light reaction of photosynthetic system, especially in salt-sensitive rice grown without root system.

B4_B0095 Estrogen Enhances the Inhibitory Effect of Iron on Microglial Nitric Oxide Production

Nootchanat Mairaue MSc, Krongkan Nasee BSc, Poonlarp Cheepsunthorn Ph.D
Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract: Abnormal iron accumulation has been consistently reported in specific brain regions of many neurological disorders. At cellular level, iron is unusually observed in microglia, immune effector cell of the brain. Most evidence has provided that upon activation, microglia produce neurotoxin and different kinds of inflammatory mediators. Therefore, it is believed that activated microglia are actively involved in neurodegenerative process. Using a rat microglial cell line (HAPI), the present study was designed to address the role of iron for immune function of microglia, in particular, the production of nitric oxide (NO) in the presence or absence of estrogen, a potential neuroprotective agent. The present results demonstrated that exposure of microglia to iron significantly decreased lipopolysaccaride-induced NO production, as determined by nitrite accumulation in the cell culture medium, and such effect of iron was potentiated by increasing concentration of estrogen. Transcript analysis revealed that estrogen, but not iron, decreased the expression of inducible nitric oxide synthase (iNOS). These results demonstrate that estrogen enhances the inhibitory effect of iron on microglial NO production by decreasing mRNA expression of iNOS and also suggest that iron sequestration by microglia under neuropathological conditions could be a protective mechanism against NO-induced neurotoxicity.

B4_B0096 Effect of auxins on calli induction and polyamines on regeneration from anther culture of indica rice (*Oryza sativa* L. *ssp indica*) varieties

Bootsaya Srianan[1], Aussanee Pichakum[1], Sittiruk Roytrakul[2], Suriyan Chaum[2] and Chalermpol Kirdmanee[2]
[1] Department of Plant Science, Faculty of Science, Mahidol University, Rama VI Rd, Payathai, Bangkok 10400
[2] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Rd, Klong Luang, Pathumthani 12120

Abstract: The effected of auxins; 2,4-Dichlorophenoxy acetic acid (2,4-D), Naphthalene acetic acid (NAA), and tryptophan on embryogenic calli induction as well as the polyamines application for plantlet regeneration were investigated. The results showed that NAA had significantly affected on embryogenic calli induction of anther culture in KDML 105. Among 3 tested varieties, the calli induction percentage of PT 1 showed the lower than those KDML 105 and HJ anther cultures. Embryogenic calli derived from anther culture were then regenerated on modified-MS media contained polyamines; 0.5 mM putrescine, spermine and spermidine. High percentage of plantlet regeneration found on 0.5 mM Put and Spd supplemented media of PT1.

B4_B0098 THE EARLY RESPONSE OF SOLUBLE SUGARS TO SODIUM CHLORIDE IN SALT-TOLERANT AND SALT-SENSITIVE INDICA RICE (*ORYZA SATIVA* L. SUB. *INDICA*)

Sarunyaporn Maksup[1], Aussanee Pichakum[1], Sittiruk Roytakul[2], Sunyan Cha-um[2] and Chalermpol Kirdmanee[2]

[1] Department of Plant Science, Faculty of Science, Mahidol University, Rama VI Rd, Payathai, Bangkok 10400

[2] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Rd, Klong Luang, Pathumthani 12120

Abstract: Soluble sugars exhibit significant roles in salt tolerance of many plants. Some evidences indicated that soluble sugars function as compatible solutes but some is conflicting. The aim of this research was to investigate the early response of soluble sugars in salt-tolerant and salt-sensitive varieties of indica rice (*Oryza sativa* L. sub. *indica*) cultured photoautotrophically under salt-stress. Rice seedlings were treated with 342 mM NaCl for 0, 15, 30, 45, and 60 min to monitor the endogenous soluble sugars, including mono-, disaccharide and sugar alcohol contents. The results showed that the changes of soluble sugars, especially sorbitol, in salt-tolerant variety; Hawm-jan rapidly responded to NaCl within 60 min. This indicated that the primary effect of sorbitol might not act as compatible solute but it may have other roles in salt tolerant mechanism.

B4_B0099 Production of Poly-β-hydroxybutyrate (PHB) by Alcaligenes eutrophus NCIMB 11599 using Cassava Starch Hydrolysate

Piyawan Boonmako and Phimchanok NAKKHARAT*

Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhon Pathom 73000, Thailand

Abstract: Poly-β-hydroxybutyrate (PHB), a biodegradable plastic, is stored as carbon and energy reservoir by a variety of microorganism. PHB is widely applied in several applications, including medicine, agriculture and industry, because of the similarities in physical properties to petrochemical plastics. The major problem associated with the production of PHB is its high production cost. The use of the inexpensive substrate, such as cassava starch hydrolysate, may help to reduce the cost of producing PHB. The objective of this work is to study the production of PHB by *Alcaligenes eutrophus* NCIMB 11599 using cassava starch hydrolysate as carbon source. The first step was to study the effect of initial glucose concentration on growth rate. The maximum specific growth rate was obtained at 0.12 h^{-1} with 15 g/l initial glucose concentration. The effect of C:N ratio on the production of PHB was also investigated. The PHB production yield reached the maximum of 0.53 g PHB/g CDW under the C:N ratio of 50. In comparison with using glucose and cassava starch hydrolysate as carbon sources, the maximum PHB concentration of approximately 5.40 and 3.10 g/l were observed, respectively. This study suggests that the use of inexpensive substrate such as cassava starch hydrolysate is one of the possibilities to reduce the high production cost of PHB production.

B4_B0101 Detections of protease activity in fruits

Apinyan Boonprakobkul[1], Sombat kongwithtaya[2], Griangsak Chairote[2], Wuttinun Raksajit[3], Surasak Laloknam[4], and Surin Thepsawat[5]

[1] Department of General Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[2] Department of Chemistry, Faculty of Science, Chiangmai University, Chiangmai, 50200, Thailand

[3] Department of General Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[4] Isotope Production Division, the Office of Atomic for Peace, Bangkok, 10900, Thailand

[5] Science Program, Satriwithaya School, Bangkok, 10200, Thailand

E-mail: duen10486@yahoo.com

Abstract: 14 kinds of samples; rose apple, pine apple, papaya, water melon, yam bean, apple, cantaloupe, mango, carrot, guava, tomato, jackfruit, orange, and pomelo were tested for proteolytic activity by using the size of clear zone around the hole. We found that all of samples showed proteolytic activity and the highest protease activity were found in mango.

B4_B0105 DIRECT ISOLATION OF NOVEL MALTOGENIC AMYLASES FROM HOT SPRING SEDIMENTS BY USING A PCR-BASED CLONING APPROACH

Kittapong Tang, Tanatchaporn Utairungsee, Verawat Champreeda, Rutchadaporn Sriprang, Lily Eurwilaichitr and Sutipa Tanapongpipat

BIOTEC Central Research Unit, National Center for Genetic Engineering and Biotechnology, Thailand Science Park, 113 Paholyothin Rd., Klong 1, Klong Luang, Paturnthani, 12120, Thailand

E-mail address: kittapong@biotec.or.th

Abstract: An attempt to isolate novel genes encoding starch-degrading enzymes directly from Bor Khleung hot spring sediment, Ratchaburi Province, was achieved in this study. Two novel genes encoding maltogenic amylases were obtained by using genome walking approach. *BK100* and *BK193* consisted of 1,761 bp and 1,818 bp that encoded 589 and 605 amino acids, respectively. BK100 exhibited 82% similarity to that of maltogenic amylase of *Bacillus acidopullulyticus* whereas BK193 exhibited 62% similarity to glycosyl hydrolase family 13 of *Salinibacter ruber* DSM 13855. Unlike BK100, BK193 harbored a putative 30-amino-acid-leader sequence. An expected size of 68 kDa protein band was observed from the supernatant of recombinant *Pichia pastoris* expressing either BK100 or BK193 by SDS-PAGE. From preliminary results, these two enzymes exhibited different substrate specificity. BK100 was able to hydrolyze α-CD, β-CD and γ-CD faster than pullulan and soluble starch whereas BK193 preferred pullulan and soluble starch to cyclodextrins. The characterizations of

both enzymes are now in progress.

B4_B0108 Metagenomic library construction and biochemical screening for lipolytic enzymes from Jae Sawn hot spring.

Pacawadee Tirawongsaroj[1], Rutchadapom Sriprang[2], Piyanun Harnpicharnchai[2], Verawat Champreda[2], Sutipa Tanapongpipat[2], Kusol Pootanakit[1] and Lily Eurwilaichitr[2]

[1] Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand.

[2] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, BIOTEC Central Research Unit, 113 Thailand Science Park, Phahonyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand.

Abstract: Lipases and esterases are used as catalysts in synthetic organic chemistry. Most of the industrial processes in which the enzymes are employed require high temperatures. Therefore, the hot spring soil sample is an attractive resource for microorganisms producing thermotolerant lipolytic enzymes. In this study, the metagenomic library of Jae Sawn hot spring was constructed and screened for lipolytic activity using both triolein and tributyrin as substrates. Among 36,000 transformants, two colonies showed lipolytic activity by producing clear halo zone around the colony. Blast search results revealed that one clone was found to encode esterase, having 36% amino acid sequence identity to an esterase of *Rhodobacter sphaeroides*, and the other clone was found to encode a phospholipase, having 39% homology to the phospholipase of *Carboxydothermus hydrogenoformans*

B4_B0117 CHARACTERIZATION OF ANTIBACTERIAL PROTEINS FROM CRASSOSTREA BELCHERI

Wirawan Nuchchanart[1], Supawadee Poompuang[1,2], Suriyan Tunkijjanukij[1,3] and Sutkhet Nakasathien[1]

[1] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakorn Pathom 73140, Thailand.; ryo_wa2000@yahoo.com Fax: (663)428-2498.

[2] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.

[3] Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand. Corresponding author: Supawadee Poompuang; e-mail: supawadee.p@ku.ac th

Abstract: The hemolymph and purified proteins of oyster *Crassostrea belcheri* exhibited significant antibacterial activity against *Vibrio spp*. The inhibitory effects of hemolymph (protein concentration of 6.363±0.923 mg/ml) against *V. harveyi, V. vulnificus* and *V cholerae* were 29.51±1.66, 22.31±1.68 and 33.38±0.42 respectively. Purified P3 protein (80 µg/ml) had strong inhibitory effects on V. parahaemolyticus (95.03±0.47), V. vulnificus (91.13±0.85), *V. alginolyticus* (86.06±1.13), *V. harveyi* (62.31±0.46) and and slight effect on *V. cholerae* (8.77±3 82). The antibacterial activity against *V. parahaemolyticus* of the P3 protein was fully effective at 30°C, pH 6-8 with 10 mM of calcium ion. Molecular weight of the protein P3 was determined by SDS-PAGE and two-dimensional electrophoresis. The protein P3 consisted of two subunits, 25.0 kDa (pI~ 3) and 30.5 kDa (pI~ 5). Amino acid sequencing of the two protein subunits were analyzed by LC-MS/MS. Comparison with nrFasta database revealed that the 25.0 kDa protein was homologous to Sarcoplasmic calcium-binding protein (SCP). The 30.5 kDa protein showed highest homology to hemocyte extracellular superoxide dismutase from Pacific oyster, *C. gigas*.

B4_B0119 OPTIMISATION OF EXPRESSION CONDITIONS OF THIOREDOXIN / AND DS-RED ORGANOPHOSPHATE HYDROLASE FUSIONS IN *Escherichia coli*

Thanaporn Laothanachareon[1], Pornpimol Sritongkham[2], Werasak Surareungchai[1], Lily Eurwilaichitr[3], and Verawat Champreda[3]

[1] Division of Biotechnology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 83 Moo 8 Bangkhuntian-Chaytalay Road Tha Kham, Bangkhuntian, Bangkok 10150, Thailand.

[2] Pilot Plant Development and Training Institute and Biochemical Engineering and Pilot Plant Unit, National Centre for Genetic Engineering and Biotechnology, King Mongkut's University of Technology Thonburi, 83 Moo 8 Bangkhuntian-Chaytalay Road Tha Kham, Bangkhuntian, Bangkok 10150, Thailand.

[3] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Road, Klong Luang, Pathumthani 12120, Thailand.

Abstract: Organophosphate hydrolase (OPH; EC 3.1.8 1) is a bacterial enzyme that catalyses the hydrolysis of ester/thioester bond of a range of organophosphate pesticides such as parathion, methyl parathion, paraoxon and coumaphos (1). In this study, the *opd* gene from *Flavobacterium* ATCC27551 was used for recombinant plasmid construction for expression of OPH in fusion forms with the highly soluble thioredoxin /and/or the red-colour protein DS-Red. The optimisation of expression conditions for the fusion proteins in *Escherichia coli* was studied. The recombinant proteins were found as optimally expressed in *E. coli* Rosetta (DE3)(pLysS) for Trx-His_6-OPH and in E. coli DH5α for His_6-DSRed-OPH after 0.5 mM IPTG induction at 18°C for 8 and 24 hours, respectively. Addition of 50 µM Co^{2+} resulted in enhancement of the enzyme activity. The paraoxon hydrolase activity was 1,277 nmole/min/mg protein and 1,712 nmole/min/mg protein for the thioredoxin and DS-Red fusion forms, respectively. The recombinant enzymes will be further used for application in detection of organophosphate pesticides in agricultural products and environment.

B4_B127 Identification and expression of cellobiohydrolase gene from an endophytic fungus, *Fusicoccum* sp. (BCC4124)

Pattanop Kanokratana[1], Duriya Chantasingh[2], Kusol Pootanakit[2] and Lily Eurwilaichitr[1]

[1] Molecular and Enzyme Screening Laboratory, BIOTEC Central Research Unit, National Center for Genetic Engineering and biotechnology, 113 Thailand Science Park, Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand.

[2] Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakhon Pathom 73170, Thailand.

Abstract: A gene encoding a cellobiohydrolase (CBH) was isolated from *Fusicoccum* sp. (BCC4124), an endophytic fungus in the phylum Ascomycota. This gene was isolated via RT-PCR using degenerate primers which was designed based on the conserved amino acid sequence of CBH from various Ascomycetes and Basidiomycetes. The full-length gene was obtained by using 5' and 3' Rapid Amplification of cDNA End (RACE) technique. The full-length cDNA encoding CBH was expressed in *Pichia pastoris*, giving a 464-amino acid protein with a molecular mass of 50 kDa. The deduced amino acid sequence showed significant similarity to those of other fungal CBH belonging to family 7 of glycosyl hydrolases. Interestingly, the result from the amino acid alignment revealed that this CBH does not contain a cellulose binding domain and a linker region. The recombinant CBH retained more than 60% of its activity after 3 h of incubation at 50°C and was stable in the pH range of 4-11. The optimal temperature was 40°C and the optimal pH was 5.

B4-B0140 Subtraction cDNA library of the gill from heat-induced Giant Tiger Shrimp, *Penaeus monodon*

Sasithon Petkon[1], Narongsak Puanglarp[1,2], Sirawut Klinbungha[1,2], Tassanee Eamkamon[1], Rungnapa Leelatanawit[1], Rachanimuk Preechapol[1], and Piamsak Menasveta[2]

[1] Center of Excellent for Marine Biotechnology (CEMB), [2]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330,

[3] National Center for Genetic Engineering and Biotechnology (BIOTEC),National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand;
e-mail: narong@biotec.or.th

Abstract: Genes expressed differentially in the gills from shrimps induced by heat at 35 °C for 4 h subtracted by that of normal shrimps and vice versa were detected using subtractive cDNA library technique. The results after comparing the resulting clones with known genes reported in GenBank using BLAST analysis revealed that, from forward subtraction, a total of 163 clones were obtained. Among these, 62 clones (38.04%) containing DNA sequences homologue to known genes were identified. Seven of them were genes reportedly involved in defense mechanisms of various animals. The remaining transcripts (101 clones, 61.96%) were identified as unknown genes which have not been previously characterized in any organisms. From reverse subtraction, a total of 167 clones were obtained. Forty-eight clones (28.74%) were analyzed as homologue to known genes. Three of them were identified as immune related genes. There were 119 clones (71.26 %) identified as unknown genes which were no match to any genes previously reported in GenBank database. Genes obtained from both forward and reverse subtraction cDNA libraries are important for the investigation on the defense mechanisms the shrimp and the potential genes will be further characterized and used as biomarkers for the determination of health status in *P.monodon*.

B4_B0142 MOLECULAR CLONING AND CHARACTERIZATION OF ESTROGEN RECEPTOR GENES IN GREENBACK MULLET (*Liza subviridis*)

Arttasit Tangserisuksan[2], Narongsak Puanglarp[1, 3*] and Piamsak Menasveta[3, 4]

[1] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand.

[2] Program of Biotechnology,

[3] Center of Excellent for Marine Biotechnology (CEMB),

[4] Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail address: narong@biotec.or.th

Abstract: Complete cDNA sequences of estrogen receptor (*ER*) α and β from a liver of estrogen-treated juvenile greenback mullet (*Liza subviridis*) were obtained. cDNA fragments of these genes were initially amplified by PCR using degenerate primers designed from ER conserved regions from other fish. 5' and 3' fragments from both α and β forms were generated by RACE-PCR using primers specific to each form of *ER*. The results revealed that the full-length *ER*α cDNA contained complete open reading frame of 1,863 bp encoding polypeptide which contained 620 amino acid residues. Molecular weight was calculated as 67.55 kDa. Full-length *ER*β cDNA contained complete open reading frames of 1,811 bp encoding polypeptide which contained 602 amino acid residues. Molecular weight was expected to be 66.22 kDa. Domains including A/B domain, nuclear hormone receptors DNA-binding domain that contained c4 zinc finger motif, D domain, hormone binding domain and F domain which are commonly found in ER from most species were all identified in deduced sequences of ERα and β. This confirms the identity of complete cDNA sequences of *ER* α and β in greenback mullet. Furthermore, these genes will be subjected to quantitative analysis on the expression level in the juvenile fish exposed to estrogen and applied as biomarkers for the determination of xenoestrogen contamination in water.

B4_B0149 ISOLATION OF GENES ENCODING NOVEL CELLULOLYTIC AND XYLANOLYTIC ENZYMES FROM TERMITE GUT USING CULTURE-INDEPENDENT APPROACH

Taksawan Thongaram, Rutchadaporn Sriprang, and Lily EurwilaichitrMolecular and Enzyme Screening Laboratory, BIOTEC

Central National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Klong 1, Klong Luang, Pathumthani 12120, Thailand

Abstract: Termites play an important role in the mineralization of complex biopolymers, such as wood and other cellulose- and hemicellulose containing materials. It has been shown that microorganisms have a significant impact on lignocellulose degradation (1). However, the cultivation-based approaches detect only a small portion of the gut microbial communities. Recent cultivation-independent studies suggest that the termite gut contains a great microbial diversity and a large number of novel, yet uncultured microorganisms (2, 3). A new approach, metagenomics, provides access to discover genes with interesting characteristics from uncultured members of microbial communities. This study includes isolation of genomic DNAs from guts of wood-feeding higher termite, *Microcerotermes* sp. and construction of metagenomic library. Since termite gut contains effective enzymes in hydrolysis of lignocellulosic materials, the obtained libraries of $2x10^5$ CFU/µg vector were constructed and screened for cellulolytic and xylanolytic activity using semi-high throughput assay. Screening of both cellulase and xylanase activity is now being in progress with fluorescence-labeled substrate. Nucleotide sequencing and comparison analysis of the obtained full-length genes responsible for cellulolytic and xylanolytic enzymes will be analyzed. Recombinant enzymes expressed in a suitable host will be further characterized.

B4_B0152 SELECTION AND MEDIA OPTIMIZATION FOR NARINGINASE PRODUCTION OF *ASPERGILLUS NIGER* PTK-BL5.1

Kanokpan Thammawat[1], Pongsuda Pongtanya[2], Vitchuporn Juntrasri[3], and Patjaraporn Wongvithoonyapom[3]

[1] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Sean Campus, Nakhorn Pathom, Thailand
E-mail address: noonbiotec_ku@yahoo.com

[2] National Center for Genetic Engineering and Biotechnology(BIOTEC), Thailand Science Park, Pathumthani, Thailand

[3] Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Sean Campus, Nakhorn Pathom, Thailand

Abstract: Three hundred forty-six fungal isolates were primarily screened for naringinase from 128 various samples, collected from 11 provinces in Thailand, by using selective solid medium. Secondary screening by naringin hydrolysis.showed that 40 isolates, out of 346, cultivating in synthetic minimal medium containing 0.1% naringin, gave good activity within 23-48 hours at pH 4.0, 40°C. Tertiary selection was based on fungi with high naringinase and α-rhamnosidase activities working at low pH but high temperature. The crude enzyme of fungus PTK-BL5.1, *Aspergillus niger*, had the optimal pH at 3.0 and 4.0 for naringinase and α-rhamnosidase, respectively whereas the optimal temperature of both activities was at 60°C. Study of the optimal conditions for naringinase production from PTK-BL5.1 in submerged fermentation was found that the suitable inoculum concentration and media were 10^6 spores/ml and Czapek Dox medium at initail pH 4.0, respectively. Moreover, 1.25 g/l rhamnose should be used as a supplement carbon source.

B4_B0153 SCREENING OF MEDICINAL PLANTS FROM KOH KRET, NONTHABURI FOR ANTIBACTERIAL ACTIVITY

Chantana Kankamol[1]*, Rinrada Promsiri[2], Ruchanok Cheotacha[1]

[1] Department of Biotechnology, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, Thailand.

[2] Departmemt of Microbiology, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, Thailand.

* Corresponding author, E-mail: fscichk@riss.ac.th, Chantanakk@gmail.com

Abstract: Forty-four extracts from eleven medicinal plants were assayed to determine their antibacterial activity against four pathogenic bacterial strains: *Staphylococcus aureus*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* and *Salmonella* Typhimurium. Almost all the extracts were able to inhibit the growth of one or more of the bacterial strains, except that of *K. pneumoniae*. The most susceptible bacterium was *S. aureus*. The highest antibacterial activity was found in extracts of *Alpinia nigra* and *Muntingia calaiura* Linn. The minimum inhibitory concentration (MICs) ranged from 1 32 to 2.64 mg/ml. The acetone extract and the ethanol extract of *Alpinia nigra* showed significant inhibition against *S. aureus* (MIC = 1.32 mg/ml). Furthermore, the water extract of *Muntingia calaiura* was also active against *S. aureus* (MIC = 1.32 mg/ml). Extracts from *Diplazium esculentum* SW. did not show any bacterial activity.

B4_B0154 CLONING, EXPRESSION AND CHARACTERIZATION OF RECOMBINANT HUMAN MANGANESE SUPEROXIDE DISMUTASE IN *ESCHERICHIA COLI*

Sakda Yainoy, Chartchalerm Isarankura Na Ayudhya, Virapong Prachayasittikul
Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.
E-mail: mtvpr@mahidol.ac.th, Fax: (662)849-6330

Abstract: Superoxide radical ($O_2^{\bullet -}$) is a toxic by–product of oxidative metabolism that extensively damages to cellular macromolecules and organelles (1). Superoxide dismutase (SOD) catalyzes the conversion of superoxide radical to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) thus providing the biological defense against oxygen toxicity (2). The structural gene of human manganese superoxide dismutase was cloned into pET46EK/LIC by using ligation independent cloning (LIC) method (3). Recombinant MnSOD was expressed in *E.coli* BL21(DE3) pLysS and was purified to homogeneity by Ni^{2+}-NTA. Mn^{2+} supplementation in the bacterial growth media was proven to be essential for production of enzymatically active rMnSOD. The recombinant enzyme revealed a specific activity up to 2857 U/mg as measured by inhibition of photoreduction

of nitro blue tetrasolium (NBT) (4). The moleculat weight of each subunit was determined to be approximately 22 kDa by SDS-PAGE. *E.coli* cells expressing rMnSOD were also shown to confer oxidative stress resistance against herbicide paraquat (5) up to 0.6 mM.

B4_B0157 PERCUTANEOUS ABSORPTIONS OF UV FILTERS

Patcharawalai Klinubol[1], Supason Wanichweacharungruang[2], Pravit Asarvanonda[3]
[1] Program of Biotechnology, Faculty of Science, Chulalonghorn University, Phyathai Road, Bangkok, Thailand.
[2] Department of Chemistry, Faculty of Science, Chulalongkorn University, Phyathai Road, Bangkok, Thailand.
[3] Department of Medicine, Faculty of Medicine, Chulalongkorn University, Phyathai Road, Bangkok, Thailand.

Abstract: In this work, percutaneous absorption of UV filters through baby mice skins(*Mus musculus* Linn.) and volunteer human skins were studied by Franz diffusion cell and Suction Blister techniques. The results showed very good correlation of the absorption result between baby mice skin and human skin. Among octyl methoxycinnamate, butyl methoxy dibenzoylmethane, 4 methyl benzylidene camphor, Eusolex UV pearl OMC, di(2ethylhexyl)-2,4,5 trimethoxybenzalmalonate, ethylhexyl-2,4,5 trimethoxycinnamate, dihexyl-2,4,5 trimethoxybenzalmalonate, diethyl-2,4,5 trimethoxybenzalmalonate, *p*-(3-hydroxy-propoxy) cinnamic acid, *p*-(6-hydroxy-hexyloxy) cinnamic acid, *p*-(11-hydroxy-undecyloxy) cinnamic acid, 4-methoxycinamoylphthaloylchitosan and 4-methoxycinamoylphthaloyl irradiated chitosan, only octyl methoxycinnamate and butyl methoxy dibenzoylmethane gave significant transdermal penetration results. The two newly developed UV filters di(2ethylhexyl)-2,4,5 trimethoxybenzalmalonate and ethylhexyl-2,4,5 trimethoxycinnamate showed no trans-epidermal penetration.

B4_B0160 ANTIOXIDANT ACTIVITIES OF NOH KALA (*Alpinia nigra* B.L. Burtt) EXTRACTS

Ruchanok Cheotacha[1*] and Chantana Kankamol[1]
[1] Department of Chemistry, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, Thailand.
[*] Corresponding author, E-mail: nu4539512@yahoo.com

Abstract: Ethanolic extracts were obtained from the rhizome, stem and leaf of medicinal plant, Noh Kala (Alpinia nigra B.L. Burtt). The antioxidant property of the extracts were investigated by two test systems, β-carotene/linoleic acid and DPPH free radical scavenging. In the β-carotene/linoleic acid test system, the ethanolic extracts from leaf and stem exhibited the highest antioxidant activities with an IC_{50} value of 12.517 and 13.260 µg/ml, respectively. For the DPPH free radical-scavenging test system, the leaf extract showed greater free radical-scavenging activities than those of other extracts studied with SC_{50} value of 0.136 mg/ml. The results of this study could be revealed that Noh Kala should be provided as a good source for antioxidant compounds.

B4_B0166 IN VITRO BULBLETS INDUCTION OF *LILIUM X FORMOLONGI*

Pheravut Wongsawad [1], Thanyalak Yongkongkaew [1], Supuk Mahadtanapuk[1], and Supaporn Kantawong[1]
[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

Abstract: The study of in vitro culture of *Lilium x formolongi*. Bulb scales were sterilized with Clorox on various concentration 10%, 20%, 30% and 40% for 20 minutes, then cultured on hormone free MS medium for 1 week. The result showed that Clorox 30% could sterile bulb scales about 62%. The explants were transferred on MS medium with BA and NAA (0, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/l respectively) for 8 weeks. The MS medium with 0.75 mg/l BA and 1.0 mg/l NAA could highly induced multiple shoot formation. After that cutting shoots into small segments were cultured on hormone free MS medium for 6 weeks and got small single shoots (6mm.). The small single shoots were transferred onto MS medium supplemented with 30, 60, 90 and 120 g/l sucrose respectively for 8 weeks. Bulblets had the biggest diameter (14mm.) on MS medium with 60 g/l sucrose.

B4_B0168 CONSTRUCTION OF CHIMERIC ANTIBODY-BINDING *VITREOSCILLA* HEMOGLOBIN (VHb) PROVIDING PEROXIDASE-LIKE ACTIVITY FOR IMMUNOLOGICAL DIAGNOSIS

Natta Tansila[1], Yaneenart Suwanwong[1], Malin Kvist[2], Chartchalerm Isarankura Na Ayudhya[1], Leif Bulow[2], Virapong Prachayasittikul[1]
[1] Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand. E-mail: mtvpr@mahidol.ac.th, Fax: (662) 849-6330
[2] Department of Pure and Applied Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden.

Abtract: Genetic construction of two chimeric genes encoding chimeric VHbs harboring one and two consecutive sequences of Fc-binding motif (Z-domain) was successfully performed and expressed in *E. coli* strain TG1. The chimeric ZVHb and ZZVHb were purified using IgG-Sepharose affinity chromatography. Binding affinity constants of the chimeric ZVHb and ZZVHb to human IgG were obtained to be 9.7×10^7 and 49.1×10^7 per molar, respectively. More importantly, the chimeric VHbs exhibited a peroxidase-like activity determined by activity staining on native PAGE and dot blotting. Effects of pH, salt, buffer system, level of peroxidase substrate and chromogen substrate were determined. The chimeric VHbs displayed their maximum peroxidase-like activity at the neutral pH (~7.0) in the presence of high concentration (20-40 mM) of H_2O_2 with the detection limit of 250 ng. All these findings on the novel functional role of the *Vitreoscilla* hemoglobin opens up a high

feasibility to further apply the chimeric VHbs in biotechnological and medical applications.

B4_B0169 An Amperometric Xanthine Oxidase Biosensor for the Determination of Tuna Fish Freshness

Orachat Apichatratanachai[1], Sopa Klinchan[2], Samorn Hirunpraditkoon[1], Boonsong Sutapan[3]

[1] Department of Chemical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology North Bangkok, Piboonsongkram Road, Bangsue, Bangkok 10800, Thailand.

[2] Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's Institute of Technology North Bangkok, Piboonsongkram Road, Bangsue, Bangkok 10800, Thailand.

[3] Department of Electrooptics, Electronic and computer center of Thailand, 112 Pahonyothin Road, Klongluang, Patumthani 12120, Thailand.

B4

Abstract: An amperometric biosensor for hypoxanthine (Hx) has been constructed and applied to the measurement of fish meat quality. The construction process involves the immobilization of xanthine oxidase in the polymetaphenylenediamine film on the surface of platinum electrode by the electropolymerization technique. Hypoxanthine was measured with the sensor by the oxidation of the enzymatic reaction product, hydrogen peroxide at optimum potentials 0.6 V (vs Ag/AgCl). The use of polymerized film of polymetaphenylenediamine as a matrix for xanthine oxidase immobilization yields enhanced specificity, sensitivity and stability. The linear of 0 mM to 0.8 mM was achieved and the response time was less than 1 minute. Satisfactory results were obtained from the determination of the freshness of tuna tissues stored under different storage time.

B4_B0170 Development of a plate technique for screening of carbohydrate-degrading enzymes

Ukrit Ratanachomsri, Piyanun Harnpichanchai, Taksawan Thongaram, Sutipa Tanapongpipat, Rutchadaporn, Sriprang and Lily Eurwilaichitr

BIOTEC Center Laboratory, 113 Thailand Science Park, Phahonyothin Road, Klong 1, Klongluang, Pathumthani 12120, Thailand. E-mail address: Ukrit@biotec.or.th

Abstract: Detection of xylanase activity based on solubilization of dye-labeled substrates and the formation of haloes on Petri dishes was investigated. We compared price, time and specificity of two screening methods. The procedures for screening were either a) direct mixing of substrate into culture medium (LB) plate followed by spreading of microorganisms on the plate and detection of xylanase activity, so-called "whole plate assay" or b) cultivation of microorganisms on LB agar followed by overlaying substrate that had been mixed with D-cycloserine before detection of xylanase activity so-called "overlayed plate assay". The substrates used in this study were Birch wood xylan, soluble dye-labeled xylan (Remazol Brilliant Blue xylan) and dye-labeled xylan (AZCL-xylan). It was found that the overlayed plate assay gave better and clearer results than the whole plate assay. The overlaying of AZCL-xylan produced the clearest, quickest and most accurate results for detection of xylanase activity. It was found that this AZCL-xylan-overlayed plate assay was 4-time cheaper than the whole plate assay. Thus, it can be used efficiently for screening of xylanase-producing microorganisms.

B4_B0171 A LOW-COST INDUSTRIAL SCALE PRODUCTION OF BACTERIORHODOPSIN FROM *Halobacterium Archea* FOR USING AS MOLECULAR ELECTRONIC DEVICES

Saipin Chaiyanan, Wimolsiri Porntaveevat, Narin Pisuthrattana, Sittikorn Theerakawong, and Surajet Jangrod

Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi, 126 Pracha-utit Road, Bangkok 10140, Thailand.

E-mail: saipin.cha@kmutt.ac.th, Fax: (662)470-8050

Abstract: Bacteriorhodopsin (BR) is a transmembrane protein of *Halobacterium*, a halophilic archaea, found in extremely high salt environment such as salt marsh. BR has recently been suggested to have several applications in the design of molecular electron devices and optical computers since it possesses desirable photophysical/chemical properties. A large quantity of BR is in demand for research. This research aimed at developing low cost BR production methods. *Halobacterium halobium*, isolated from Samutsakorn province sea salt, was used in the study. We examined the effect of light, temperature, and aeration on growth and BR production of the *H. halobium* in the standard culture medium, Halobacterium medium. The maximum cell growth and BR production were detected when *H. halobium* was culture in light at 37 °C with shaking at 200 rpm. In this study, we attempted to grow *H. halobium* using liquid waste from sea food processing to replace high price yeast extract and peptone in the culture medium. The results demonstrated that liquid waste from salting anchovies industry could be used in cultivation and reduced the cost of medium up to 96%.

B4_B0173 CHARACTERIZATION AND PURIFICATION OF XYLANASE FROM *TRICHODERMA KONINGII* (BCC4555) ISOLATED FROM THAILAND

Warasirin Sornlek, Ukrit Rattanachomsri, Benchaporn Buaban, Rutchadaporn sriprang, Sutipa Tanapongpipat, and Lily Eurwilaichitr

BIOTEC Central Research Unit 113 Thailand Science Park, Klong Luang, Pathumthani 12120

E-mail address: warasirin@biotec.or.th

Abstract: *Trichoderma koningii*, (BCC4555) from BIOTEC culture collection (BCC) was subjected to study for xylanolytic enzyme production as well as enzyme purification and characterization. This fungal strain produced high yield of xylanase in both WS medium (3% wheat bran and 1% soybean) and MX medium (1% xylan and basal salts). We successfully purified

xylanase from both sources of enzyme. The xylanase from WS medium was purified by single step of FPLC using anion exchange column (Q-sepharose) giving protein with a molecular weight of 30 kDa (XynA). While xylanase (XynB) from MX medium with a molecular weight of 20 kDa was purified by two consecutive columns (anion exchange column followed by gel filtration column). The XynA showed the optimal pH of 5 and optimal temperature of 60°C. When tested towards Birchwood xylan it showed K_m value of 2.4±0.3 mg/ml (k_{cat} = 154.5 sec^{-1}). Furthermore, the purified XynA demonstrated broad pH stability from 3-8 and moderate thermal stability (retained about 74% of its activity when incubated at 50 °C, 2 hours). The XynB showed the optimal pH of 5 and optimal temperature of 50°C. When tested towards Birchwood xylan it showed K_m value of 2.2±0.3 mg/ml (k_{cat} = 534.0 sec^{-1}).

B4_B0180 CHARACTERIZATION OF A PHOSPHOLIPASE ISOLATED FROM JAE-SAWN HOT SPRING

Pacawadee Tirawongsaroj[1], Piyanun Harnpicharnchai[2], Rutchadaporn Sriprang[2], Verawat Champreda[2], Sutipa Tanapongpipat[2], Kusol Pootanakit[1] and Lily Eurwilaichitr[2]

[1] Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand.

[2] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, 113 Thailand Science Park, Phahonyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand.

Abstract: Lipases are used as catalysts in synthetic organic chemistry and various important industries. In this study, a phospholipase containing lipolytic activity (capable of using both triolein and tributyrin as substrates and producing clear halo zone around the colony) was isolated from 36,000 transformants of the metagenomic library of Jae-sawn hot spring. The identified phospholipase has predicted molecular weight of 32 kDa and is encoded by a gene with 873 nucleotides. The enzyme contains 39% homology to the phospholipase of *Carboxydothermus hydrogenoformans*. This phospholipase can function well at high temperature with optimal temperature of 50°C. At 60°C, the enzyme exhibits 78% of its maximal activity. The optimal pH for this enzyme is 8-9. In addition, the enzyme can hydrolyze *p*-nitrophenyl valerate better than *p*-nitrophenyl butyrate, when appearance of *p*-nitrophenyl was used as indicator of phospholipase reaction. Unit activity and specific activity toward various substrates are being investigated. Thermostability of the enzyme is being investigated.

B4_B0184 LIGNOLYTIC ACTIVITIES AND MOLASSES PIGMENTS DECOLORIZATION CAPABILITY OF *GANODERMA FORNICATUM* GF

Solaya Suksa-ard[1], Chawalit Hongprayoon[2], Malee Srisodsuk[3] and Wichai Kositratana[1,4]

[1] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen, Nakhon Prathom, Thailand.

[2] Department of Soil Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Prathom, Thailand.

[3] Department of Microbiology, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen, Nakhon Prathom, Thailand.

[4] Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Prathom, Thailand.

Abstract: Lignins constitute the second most abundant group of biopolymers in the biosphere. It is structurally complex aromatic biopolymer which is recalcitrant to degradation. Lignolytic enzymes play important roles in depolymerization of lignin and a broad spectrum of structurally different substrates including highly toxic phenolic compounds, azo dyes and pigments. White-rot basidiomycetes produce various isoforms of ligninolytic enzymes including manganese peroxidase, lignin peroxidase and laccase. In this research, lignolytic basidiomycete, *Ganoderma fornicatum* GF was grown on 40-60 mesh *Eucalyptus* powder for 8 weeks and lignolytic activities were determined against known lignolytic basidiomycete *Trametes hirsuta* YK505. It was found that lignolytic activities of GF were similar to YK505. The highest manganese peroxidase activity of GF was 95 U/l within six weeks while lignin peroxidase activity was 21 U/l within four weeks. Decolorization of 2.5% w/v molasses pigments of GF was found to be higher than YK505. The mushroom exhibited high tendency for further application in delignification and decolorization.

B4_B0186 PHYSIOLOGICAL, GROWTH AND YIELD RESPONSES OF TRANSGENIC COTTONS TO WATERLOGGING IN THE FIELD

Pongmanee Thongbai[1], Steve Milroy[2], Mike Bange[2] and Marc Ellis[2]

[1] School of Science, Mae Fah Luang University, A. Muang, Chaing Rai, Thailand.
E-mail:pongmanee@mfu.ac.th, Fax: (6653)916776

[2] CSIRO Cotton Research Unit, Narrabri, NSW 2390, AUSTRALIA.

Abstract: Most of the Bt cottons provide high yield in the optimal growing conditions. Waterlogging, to which cotton is very susceptible, could affect growth and yield considerably. Objectives of this research is to investigate how photosynthesis, growth, development and yield of the field-grown Bt cotton responding to waterlogging as well as testing some possible mechanisms involved. Biomass and yield of waterlogged cotton decreased and significantly correlated with soil O_2 content. Yield reduction was due to overall growth reduction and thus total boll number, not from squares and boll shedding. Waterlogging also reduced photosynthesis rate, intercepted photosynthetically active radiation (PARi) and radiation use efficiency (RUE). Yield of the six transgenic cottons over-expressing the genes controlling alcohol dehydrogenes (ADH) and pyruvate decarboxylase (PDC) were also non significantly different from the non-transgenic control. These results suggested that genictically modified alcoholic fermentation process might not be the only solution to increase waterlogging tolerance in cotton.

B4_B0187 Larvicidal activity of germinated conidiospores of *Beauveria bassiana* (BCC2659) against Lepidoptera larva, *Spodoptera exigua*

Duriya Chantasingh[1,2], Kusol Pootanakit[2] and Lily Eurwilaichitr[1]

[1] BIOTEC Center Laboratory, 113 Phahonyothin Road, Klong 1, Klongluang, Pathumthani 12120, Thailand.

[2] Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Salaya, Phuttamonthon District, Nakhon Pathom 73170, Thailand.

E-mail address: duriya@biotec.or.th

Abstract: Entomopathogenic fungi have been developed to control insect pest for more than a decade by using fungal conidiospore as an active ingredient. However, using germinated conidia in pest control was never investigated. Here, we demonstrated the ability of *Beauveria bassiana* germinated spore in the killing of *Spodoptera exigua* larvae. In the beginning, *B. bassiana* conidiospores development was conducted in Sabouraud maltose broth. Germination reached 50% and 100% at 13 and 24 hours after cultivation. Germinated spores were subsequently used in insect bioassay against fourth instars larvae of *S. exigua*. At 48 and 72 hours post-exposure, larval mortality from the 50% and 100% germinated spore exposure was significantly higher than that of non-germinated spore exposure. The results suggested that the pre-germinated spore has a higher larvicidal activity than the non-germinated spores. This study will lead to the investigation of molecular factors in the germinated spores that involve fungal virulence and pathogenicity.

B4_B0188 THE FEASIBILITY STUDY OF BIO-ETHANOL PRODUCTION FROM RICE CROP BY-PRODUCTS

Nareerat Moonjai, Kanokvan Srakaew and Janjira Salubchua

Department of Biological Sciences, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

E-mail: nareerat@sci.ubu.ac.th

Abstract: The ethanol production from lignoellulosic rice by-products was studied. The raw materials were enzymatic hydrolyzed with *Trichoderma* sp. T-11 either in submerged fermentation (SmF) or solid-state fermentation (SSF) conditions. The fermentation with *Saccharomyces cerevisiae* was started right after the enzymatic hydrolysis. It was found that SSF gave higher ethanol yield than with SmF. In the SSF, ethanol yields between 0.02-0.04 g/g substrate were recorded when using rice bran or/and rice polish. The yields were increased by 2 and 3 times when using rice broken together with rice polish and rice bran, respectively.

B4_B0199 Effects of preculture on jackfruit cryopreservation by vitrification

Witayaporn Pornchuti[1] and Kanchit Thammasiri[2]

[1] Department of Biotechnology, Faculty of Science, Mahidol University

[2] Department of Plant Science, Faculty of Science and Institute of Science and Technology for Research and Development, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

Abstract: Cryopreservation of jackfruit (*Artocarpus heterophyllus* Lamk.) in liquid nitrogen by vitrification was studied. Cryopreserved embryonic axes were able to develop into the whole plantlets. The optimal conditions were precultured embryonic axes for 3 d in MS liquid medium supplemented with 0.4 M sucrose plus 2.0 M glycerol. For vitrification, the embryonic axes were loaded with 0.4 M sucrose plus 2.0 M glycerol MS liquid medium for 20 min and exposed to plant vitrification solution 2 (PVS2) for 150 min at 25 ± 2 °C. Then the embryonic axes were plunged into liquid nitrogen. After thawing at about 40 °C for 2 min, embryonic axes were unloaded with MS liquid medium containing 1.2 M sucrose for 20 min and cultured on recovery medium (MS agar medium with 5 mg/l 6-benzylaminopurine). The survival was about 50%. According to the morphology and the Amplified fragment length polymorphism (AFLP) profiles of regenerated plantlets, the genetic fidelity of the cryopreserved jackfruit materials were the same as non-cryopreserved ones.

B4_B0204 Diversity of Plant in Ubon Ratchathani University

Assistant Professor Kaew Udomsirichakorn* and Dr. Thavorn Supaprom*

* Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

Abstract: The forests in Ubon Ratchathani University (UBU) were surveyed and collected during June 2004 – June 2005. It was found that the previous native forests that had ever been characterized as dry evergreen, dipterocarp and mixed deciduous forests was changed to scrub forest due to the former problem of deforestation.. The plants that are mostly found are pioneer plants mixed with the native plants. The forests, however, seem to be restored to its former biodiversity. After the unique characteristics of plants, forty five families, 90 genera, and 100 species are enumerated and divided into 2 groups. The first is the naked seed plant, of which 1 family, 1 genus, and 1 species has been recorded. The second is the dicotyledonous plants, which comprise 44 families, 89 genera, and 99 species. The plants that are found the most are the Leguminosae, the Rubiaceae and the Dipterocarpaceae families, respectively.

B5_B0012 Purification by molecular filtration and determination of some biochemical properties of the 42 kDa biotin-coupled proteins from chick retina and brain.

Khomkrit Arunchaipong[1], Jintana Sattayasai[2], Suporn Nuchadomrong[3], and Nison Sattayasai[3]

[1] Graduate School, Khon Kaen University, Mittraphab Road, Khon Kaen 40002, Thailand. E-mail: rainheart_eak@yahoo.com, Tel: +66-43-342911, Fax: +66-43-342911

[2] Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

[3] Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon kaen, Thailand.

Abstract: Glutamate (Glu) is an excitatory amino acid and primarily neurotransmitter in retina and brain. However, Glu also has neurotoxic effects on retina's neural cells. Apart from many reports on toxicity of Glu on retinal neurons, some retinal proteins were affected by Glu. One of these proteins, which was biotin-coupled, had molecular weight of 42 kDa. In this research, the 42 kDa proteins which were a major protein in retina but a minor protein in brain, were purified by using molecular filtration in the presence of sodium chloride solution. The purified 42 kDa proteins from both organs were tested for some biochemical properties and found to be biotin-coupled. Numbers of the 42 kDa protein's native complexes were examined by native-PAGE and evaluated for molecular weights by Ferguson plot. The results obtained were the same for the 42 kDa proteins purified from both retina and brain tissues. The results showed two native complexes of about 205 kDa and 152 kDa which were corresponded to 5 and 4 subunits of this 42 kDa proteins, respectively.

B5_B0018 PRODUCTION OF PROTEOLYTIC ENZYME FROM BACTERIA IN PEAT SWAMP FOREST

Chaowaneeporn Cheppraseop[1] and Saowanit Chobbun[2]
[1] Program of Chemistry, Faculty of Science and Technology, Songkhla Rajabhat University, Songkhla 90000
[2] Program of Biology, Faculty of Science and Technology, Songkhla Rajabhat University, Songkhla 90000
chaowaneeporn@yahoo.com

Abstract: *Bacillus* sp. psw17 and *Bacillus* sp. psw59 isolated from soil sediment in Sinnthon Peat Swamp Forest, Narathiwat Province, Thailand. They grew optimally at temperature of 30-60°C, at pH 4.0-5.0 and pH 8.0-9.0, respectively. Both of bacteria produced extracellular proteolytic enzyme in skimmed milk medium at pH 5.0, 60°C and pH 8.0, 50°C, respectively. They exhibited the substrate specificity on azocasein and casein. Moreover, the semi-purified enzyme was precipitated from supernatant of *Bacillus* sp. psw17 and *Bacillus* sp. psw59 by 80% ammonium sulfate showed the highest activity at pH 4.0, 70°C and pH 9.0, 60°C, respectively.

B5_B0020 DETERMINATION OF BIOGENIC AMINES CONTENTS IN SOM-FUG BY ENZYMATIC METHOD.

Rungnapa Chotongdee, Jirasak Kongkiattikajorn and Khanok Ratanakhanokchai
School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand.

Abstract: Bromoperoxidase (BPO) from seaweed *Gracilaria changii* was used to determine the biogenic amines contents in Som-fug by enzyme coupling assay with diamine oxidase (DAO). Biogenic amines were detected from a colored reaction product from bromination of color reagent RA, substrate of BPO, and hydrogen peroxide from DAO catalyzed deamination with biogenic amines. The optimal pH and temperature of crude BPO and DAO in the enzyme coupling assay using putrescine, cadaverine and histamine as substrates were 6.5-7.0 and 45-55 °C, respectively. Seven commercial brands of unexpired Som-fug were analyzed for the biogenic amines contents by enzyme coupling assay of DAO and crude BPO under suitable conditions. The Som-fug samples were kept at 4 °C. Different Som-fug samples showed different contents of the biogenic amines in the range of 85-158 mg/Kg, which were considered not dangerous for health.

B5_B0024 CYTOTOXICITY AND APOPTOSIS INDUCTION ACTIVITIES OF ESSENTIAL OIL EXTRACTED FROM LEMON GRASS (*Cymbopogon citrates* (DC ex Nees) Stapf.)) ON KB AND HeLa CELL LINES

Jiradej Manosroi[1,2], Pongsathorn Ohumtanom[1], and Aranya Manosroi[1,2]
[1] Natural Products Research and Development Center (NPRDC), Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai, Thailand
[2] Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
E-mail address; pmpti006@chiangmai.ac.th

Abstract: The objective of this study was to investigate the cytotoxicity and apoptosis induction activities of essential oil extracted from Lemon grass (*Cymbopogon citratus* (DC ex Nees) Stapf.) on KB (human mouth epidermal carcinoma) and HeLa (human cervical adenocarcinoma) cell lines. Lemon grass oil at the concentration of 0.05-5.0 mg/ml were incubated with the cells in 96-well tissue culture plate for 24h and cytotoxicity was detected by MTT assay. IC_{50} values of Lemon grass oil on KB and HeLa cell lines were 0.115 and 0.063 mg/ml which were 1.44 and 5.63 times stronger than vincristine (positive control), respectively. Induction of morphological changes, apoptotic bodies and single large vesicle, were observed at the oil concentration of 2.13 mg/ml after 1h of exposure on both cell lines. Over 90 % of apoptotic bodies were demonstrated, whereas the rest were single large vesicles. Low molecular weight DNA fragments were appeared at oil concentration of 0.034 mg/ml after 24h incubation on both cell lines. The results from this study can be applied for further development of Lemon grass for pharmaceutical and/or neutraceutical applications.

B5_B0025 EFFECT OF AEROBIC EXERCISE ON SERUM CAROTENOIDS

Chutima Sinlapavech[1], Smartachai Santawanpas[2]
[1] Biological Science Program, Faculty of Science, Burapha University
[2] Department of Biochemistry, Faculty of Science, Burapha University

Abstract: Regular physical exercise has a number of beneficial effects. But physical exercise may cause an oxidative stress which can lead to many disease. The purpose of this research was to study the effect of aerobic exercise on serum carotenoids concentration, which is a group of antioxidant. Thirty-eight male subjects were divided into two groups; eighteen

sedentary and twenty regular exercise people. The subjects had to exercise on a cycle ergometer, warm up at 50% maximal heart rate for 3 min then increase intensity to 60-70% maximal heart rate for 10 min and decrease intensity to 50-60% maximal heart rate until 30 min. Blood samples were collected before and after exercise. Total carotenoids in serum was determined spectrophotometically at 450 nm. β-Carotene, β-cryptoxanthin, lutein, and lycopene in serum were detected by HPLC. It was found that aerobic exercise had no effect on serum carotenoids level. It may be concluded that during aerobic exercise, the free radicals produced might be destroyed by other system of antioxidants.

B5_B0028 EXPRESSION OF CHITINASE FROM LEUCAENA LEUCOCEPHALA DE WIT IN DAWK MALI 105 AND KOSHIHIKARI

Mana Kaomek[1] and James R. Ketudat-Cairns[2]

[1] Science Center, Valaya Alongkorn Rajabhat University, Pathumthani, Thailand,

[2] School of Biochemistry, Institute of Science, Suranaree University of Technology, Muang District, Nakorn Ratchasima, Thailand. E-mail address: mana@vru.ac.th, cairns@ccs.sut.ac.th

Abstract: The objective of this research is study the expression of chitinase from *Leucaena leucocephala* de Wit in Dawk Mali 105 and Koshihigari by agrobacterium with pCAMBIA 1300. Callus of Khao Dawk Mali 105 and Koshihigari were produced and transformed with agrobacterium with pCAMBIA 1300 + NOS + chitinase and promoter CaMV35S. After that, they were checked the expression of chitinase and gus by PCR, Northern blot gel, Southern blot gel and gus assay. Chitinase and gus shown to express in all part of two rice, such as callus, leave, root, and seedling but they shown little expression in root of two rice. For antifungal of rice chitinase, they showed antifungal the *Fusarium monoliforme*.

B5_B0031 EFFECT OF CARBOXY-PTIO ON HEME OXYGENASE-1 EXPRESSION IN RAW 264.7 MACROPHAGES.

Klaokwan Srisook[1] and Nadtaya Mongkol[2]

[1] Department of Biochemistry, Faculty of Science, Burapha University, Chonburi, Thailand. E-mail: klaokwan@buu.ac.th

[2] Biological Science Program, Faculty of Science, Burapha University, Chonburi, Thailand.

Abstract: Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that degrades heme to ferrous iron, carbon monoxide (CO), and biliverdin. HO-1 is induced by various stimulants including nitric oxide (NO). Carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO) is a specific NO scavenger and has been shown to attenuate hypertension in septic shock animal models. Here, we investigated the effect of carboxy-PTIO on the induction of HO-1 expression in RAW 264.7 macrophages by employing RT-PCR and Western blotting techniques. In macrophage cells exposed to carboxy-PTIO, expression of HO-1 mRNA and protein was increased in a dose- and time-dependent manner. Viability of cells treated in various concentrations of carboxy-PTIO was determined by employing MTT test. Exposure to the high dose of carboxy-PTIO (500 μM) for 12 h was cytotoxic to macrophages, while the lower doses (10 to 200 μM) had no effect to cell survival. Furthermore, the up-regulation of HO-1 expression by carboxy-PTIO was markedly abolished by a co-incubation of the cells with well-known antioxidants N-acetyl-L-cysteine (NAC) and Trolox. These results indicated that carboxy-PTIO might regulate HO-1 expression through the ROS formation due to direct scavenging action against NO.

B5_B0032 Production of the truncated fragments of the Bacillus sphaericus binary toxin in Escherichia coli

Patcharee Promdonkoy[1], Sakol Panyim[2] and Boonhiang Promdonkoy[1]

[1] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120 and [2]Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakhonpathom 73170
Email address: patchareepr@biotec.or.th

Abstract: Binary toxin from *Bacillus sphaericus* is composed of two components; BinA (42 kDa) and BinB (51 kDa). Naturally, the toxin is produced as a protoxin which is inactive. After ingestion by susceptible larvae, the protoxin will be solubilized and finally activated by gut proteases at both N and C-termini yielding the active toxin. The active fragment could be obtained in vitro by digestion with protease but the toxin is usually unstable and degraded. In order to produce the active toxin without protease digestion, genes encoding truncated BinA (amino acid 11-353) and truncated BinB (amino acid 33-408) were cloned in expression vectors to express as a non-fusion and GST-fusion proteins. The proteins were highly expressed in *E. coli* and showed high toxicity comparable to the wild type. These results indicated that the active fragments of both BinA and BinB could retain their biological and biochemical properties similar to that of the wild type. These fragments could be used to study the mechanism of the toxin *in vitro* without protease digestion.

B5_B0033 Effects of mutation in the Aglycone-binding pocket of THAI ROSEWOOD β-GLUCOSIDASE.

Penporn Sujiwattanarat[1], M.R. Jisnuson Svasti[2], Prachumporn Kongsaeree[3]

[1] Interdisciplinary Graduate Program in Genetic Engineering, Faculty of Graduate School, Kasetsart University, Bangkok 10900 Thailand.
E-mail:beer_penporn@yahoo.com, Fax: (662) 561-4627

[2] Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok 10400 Thailand

[3] Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900 Thailand

Abstract: Dalcochinase, a β-glucosidase from Thai rosewood, can hydrolyze dalcochinin β-glucoside that is its natural

substrate, whereas linamarase, a β-glucosidase from cassava, hydrolyzes its natural substrate linamarin. Dalcochinase can catalyze reverse hydrolysis well, but shows low efficiency in transglucosylation. On the other hand, linamarase catalyses transglucosylation better than dalcochinase, but was not efficient in catalyzing reverse hydrolysis. Despite these differences, both enzymes have 60% amino acid sequence homology. Thus, this project is interested in studying the relationship between structure and function of β-glucosidase, particularly the identification of the amino acid residue that is important for hydrolysis and transglucosylation. The coding sequence of dalcochinase was cloned, expressed in yeast *Pichia pastoris*, and purified. The recombinant enzyme exhibits similar enzymatic properties to natural dalcochinase. Mutant forms of dalcochinase (namely N189F and A454N) were generated by replacing amino acid residues located in the aglycone binding pocket of dalcochinase with the corresponding residues of linamarase. Kinetic analysis of both enzymes showed that both N189 and A454 were not involved in hydrolysis of linamarin, but N189 could be important for hydrolysis of *para*-nitrophenyl-β-D-glucoside and dalcochinin β-glucoside. In transglucosylation studies, N189F mutant could improve transglucosylation efficiency using primary alcohols as acceptors. However, neither N189 nor A454 was likely to be involved in transglucosylation using secondary and tertiary alcohols as acceptors.

B5_B0035 THE ROLE OF PUTRESCINE FOR OSMOREGULATION IN CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803

Wuttinun Raksajit, Surachet Burut-Archanai, Wipawee Baebprasert and Aran Incharoensakdi
Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
E-mail: ipuento@yahoo.com, Fax: (662)218-5418

Abstract: The transport of putrescine into a moderately salt tolerant cyanobacterium *Synechocystis* sp. PCC 6803 was characterized by measuring the uptake of radioactively-labeled putrescine. Slow growth was observed in cells grown under salt stress (550 mM NaCl). Addition of 0.50 mM of putrescine could restore growth almost to the level of that without salt stress. Putrescine transport showed saturation kinetics with an appearent K_m of 98 μM and V_{max} of 0.33 nmol min^{-1} mg protein $^{-1}$. Upshift of the external osmolality generated by either NaCl or sorbitol caused an increased putrescine transport with an optimum 2-fold increase at 20 mosmol kg^{-1}. The stimulation of putrescine transport mediated by osmotic upshift was abolished in chloramphenicol-treated cells, suggesting the possible involvement of an inducible transport system.

B5_B0036 NITRATE AND PHOSPHATE UPTAKE BY CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803 UNDER NON-STRESS AND SALT-STRESS CONDITIONS

Wipawee Baebprasert, Surachet Burut-Archanai, Wuttinun Raksajit and Aran Incharoensakdi
Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
E-mail: wbaebprasert@yahoo.com, Fax: (662)218-5418

Abstract: The transport of nitrate and phosphate into a moderately salt tolerant cyanobacterium *Synechocystis* sp. PCC 6803 was determined. Increasing the concentration of NaCl up to 350 mM resulted in the reduction of the growth rate of the cells. Under nitrate deficiency, this cyanobacterium could not survive, while phosphate deficiency resulted in the acclimation of the growth rate of the cells after 3 days. Upshift of the external osmolality generated by NaCl decreased nitrate and phosphate transport with a 2-fold decrease at 685 mosmol kg^{-1}, suggesting that the depending of nitrate and phosphate transport an external salinity.

B5_B0037 CLONONG AND EXPRESION OF A GLYCINE-RICH-SINGLE WHEY ACIDIC PROTEIN-DOMAIN (GWAP) (GWAP) ANTIMICROBIAL PEPTIDE FROM BLACK TIGER SHRIMP *Penaeus monodon*

Piti Amparyup[1,2], Ikuo Hirono[3], Takashi Aoki[3] and Anchalee Tassanakajon[1]
[1] Shrimp Molecular Biology and Genomics Laboratory, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[2] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Rd., Klong1, Klong Luang, Pathumthani, 12120, Thailand
[3] Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku 108-8477, Japan

Abstract: The full-length cDNA of a glycine-rich-single whey acidic protein-domain (GWAP) antimicrobial peptide from hemocytes of black tiger shrimp, *Penaeus monodon* was cloned and characterized by 5′ rapid amplification cDNA end (RACE). The complete cDNA sequence of 541 bp contains an open reading frame (ORF) of 426 bp encoded a 141 amino acid protein including a 17 amino acid signal peptide. The calculated molecular mass of the mature protein (124 amino acid) is 12.48 kDa with an estimated pI of 7.8. This peptide exhibited a Gly-rich domain and a Cys-rich domain containing a single whey acidic protein (WAP) domain at the *N*-terminus and the *C*-terminus, respectively. The mature GWAP coding sequence was cloned into the pET28b expression vector and was highly expressed in *E. coli* BL21 codon plus with IPTG induction. The expressed protein, fused to His-tag, in the insoluble form was purified by Ni-NTA chromatography. Western blot analysis of the purified proteins revealed successful expression of rGWAP. Expression patterns of a GWAP transcript in different tissue and different developmental stages of *P. monodon* was examined by RT-PCR. The result revealed that a GWAP was expressed in all developmental stages. Tissue distribution analysis indicated that GWAP was abundantly expressed in hemocyte followed by eyestalk, gill and intestine but this transcript was not expressed in hepatopancreas, lymphoid and heart.