

## Abstracts of the Papers Presented at the 20th Biennial Conference of the AABE

The 20th Biennial Conference of the AABE was held at Chiang Mai University, Chiang Mai, Thailand from 26 to 30 December, 2004. The theme of the Conference was "Role of Modern Technologies in Biology Education." There were two plenary lectures, ten oral presentations and 19 poster presentations. In addition, two country reports from Korea and Japan were also presented.

<Plenary Lecture>

### **Bee Biology for Bio-education**

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Biology of the honeybee is the good subject for biology education in all level from elementary school to the university. The honeybee group is a social insect that can survive in a wide range of environments because of its ability to store and preserve surplus food in the form of honey. This surplus honey has been harvested by humans since ancient times. Today, large-scale the manipulation of honeybee colonies occurs in most parts of the world, providing honey, beeswax and other hive products. Yet by far the greatest benefit of the honeybee is the pollination of a wide range of agricultural and horticultural crops. The overview of honeybee biology, aims to give an insight into the biological concepts behind the practical techniques of apiculture and the other research from molecular biology level to the other advance biology especially in the Ethology (Behavior Science) subject. Prof. Karl von Frisch had received the Nobel Prize in the year 1973 in this field.

Four of the nine species of honeybees; *Apis cerana*, *A. florea*, *A. andreniformis* and *A. dorsata* are native

to Thailand, *Apis mellifera*, the European honeybee, a commercial species, was first introduced into Thailand in the early 1940s for research purposes at Chulalongkorn University. Now, this species is preferred for beekeeping all over the world. More than 100,000 colonies exist in the northern regions, and about 200,000 colonies exist throughout Thailand. Honeybees gather their food, pollen and nectar, from flowers. Pollen provides bees with the building blocks of life: amino acids, vitamins minerals and lipids (fats and their derivatives). Nectar provides energy in the form of simple sugars, which are processed by the bees and stored in the hive as honey.

**Key words:** *Apis*, biology education, ethology, honeybee.

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<Oral Presentations>

### **Genetic Literacy Education Programs Using the Internet**

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Our school is specified as a SSH (Super Science High school) of the Ministry of Education, Culture, Sports, Science and Technology in Japan, and is performing the experiment program centering on life science to high school students (10th grade) this year.

#### ***Genetic literacy educational program***

One of the SSH program's aims is giving the genetic literacy to students through the experiments using pGLO Bacterial Transformation Kit, Green Fluorescent Protein Purification Kit (BIO-RAD

Biotechnology Explorer™ Kits), and the PCR (Polymerase Chain Reaction) method for Genetic diagnosis.

#### **Information educational program**

Our school has been giving each student a notebook PC since they entered junior high school, and is focusing on information education. Recently, they are now enjoying the spread of the Internet in Japan. The Websites using ADSL (Asymmetric Digital Subscriber Line) are common at many homes. I made a SSH's special Website on the Internet and this Website is used for the preparation of those experiments, and the review and the questions after an experiment was performed. The newly developed programs mentioned above are reported.

**Key words:** genetic literacy programs, information education program, Internet, Super Science High school.

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### **Recent Advances in Kinema Research**

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Kinema is a non-salted, solid-substrate, natural fermented flavoursome food, traditionally consumed by a large section of the people of eastern Himalayas. It serves as a tasty low-cost protein complement to rice. Kinema production is still largely a household art practiced in a crude manner. This fish or meat analogue is similar to Burmese chine pepoke, Thai thua-nao, Chinese schuidouchi, Korean chungkukjang, Japanese natto and West African dawadawa. Fresh kinema of good quality has a nutty flavour accompanied by a smell of ammonia, a greyish brown colour and is semi-hard like raisins. After a brief frying in oil, kinema is added with vegetables, spices, salt and a little amount of water to prepare a thick curry. *Bacil-*

*lus subtilis* is the predominant microorganism in kinema, and also responsible for its production. The accompanying flora is probably opportunistic. A number of interesting changes occur during fermentation of soybeans. In monoculture fermentation, the count of *Bacillus* cells increases from an initial log 5 per gram wet soybeans to log 10 after 48 h at 37°C. This leads to a more desirable fermentation compared with the natural fermentation. The pH drops from an initial 6.9 to about 6.4 after 8 h and then rises to 8.6 at the end of fermentation, with a coincident increase in proteolytic activity. The free amino acid content of unfermented soybeans is only 0.2% of the total dry mass, but increases 60-fold in kinema. The major fatty acids are liberated during fermentation by microbial lipases. The unsaponifiable soybean lipids increase by 50-61% in kinema. The B-group vitamins increase significantly. Kinema contains no detectable soy oligosaccharides, which are a potential cause of flatulence in humans. Although commercial kinema often contains toxicogenic strains of bacterial contaminants, its culinary use in curries is safe. For its wholesomeness, kinema is a prospective food item in the diet of people, particularly the vegetarians. The results create a basis for improvement of processing technology to obtain reproducible high-quality kinema with elevated nutrient level.

**Key words:** *Bacillus subtilis*, eastern Himalayas, fermented food, kinema, nutrients analysis, soybean.

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### **An Ideal Laboratory Experiment on Flower Initiation**

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For a student laboratory exercise on flower initia-

tion (floral development) under the topic of “Plant Growth and Its Regulation” in senior high school biology, we selected the Wisconsin Fast Plants (WFPs), the new varieties of rape (*Brassica campestris* or *B. rapa*) which have been developed by Professor Williams of the University of Wisconsin, as our experimental material. It has been reported that under the optimal conditions, the flower-opening of WFPs is observed 14 days after sowing, the height of a mature plant is very short (less than 20 cm) and the plant completes its life cycle within 5 weeks. It seems that WFPs might be an ideal material, as a long-day plant, to study flower initiation. So, we examined the effects of temperature, day length, light intensity and the plant hormone gibberellic acid (GA<sub>3</sub>) on the flower initiation of the WFPs. When the plants were grown at different temperatures under continuous light, the days required for detecting a floral bud were dependent on the growth temperature being between 15°C and 35°C; the higher the temperature is, the faster the flower bud formation is. In the temperature range examined flower bud could be detected within at least 9 days after sowing. The effects of day length on flower bud formation were examined at 15°C and 25°C. The flower initiation was dependent on the day length at 15°C, but not at 25°C. At 15°C, flower buds formed 9 days after sowing under continuous light, while no flower bud was detected nearly 2 weeks after sowing under a short-day (L:D=8:16) condition. The intensity of light in a long-day treatment (L:D=16:8) from about 10  $\mu\text{mol}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$  (ca. 1,500 lux) to 100  $\mu\text{mol}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$  (ca. 13,000 lux) at 15°C did not affect flower bud formation. The flower initiation in the plants treated with 0.01  $\mu\text{g}/\text{mL}$  GA<sub>3</sub> was faster than in the non-treated plants. The results obtained in the present study reveal that by using the WFPs students can get clear results on flower initiation within shorter period than by using any other plant.

**Key words:** *Brassica campestris*, day length, flower initiation, gibberellic acid, student labora-

tory, Wisconsin Fast Plants.

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### **Effects of Vitamin B<sub>6</sub> and Yeast Deficiency on Nile Tilapia, *Oreochromis niloticus* Linn.**

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Effect of Vitamin B<sub>6</sub> and yeast deficiency on growth, mortality and deformities on Nile Tilapia Juvenile was studied. Nile Tilapia Juvenile average weight 0.65 grams were fed by two experimental diets. The two diets were completely vitamin control diet and yeast (formula 1 diet) and vitamin B<sub>6</sub> and yeast free diet (formula 2 diet). These experiments were conducted within 8 weeks. The results showed that fish fed by formula 1 diet grew faster ( $p < 0.05$ ) than fish fed by formula 2 diet. Mortality of fish fed by formula 2 diet was significantly higher ( $p < 0.05$ ) than fish fed by formula 1 diet obviously. In the fourth week, the symptoms of fish fed by vitamin B<sub>6</sub> and yeast free diet were similar to those reported in other fishes, fish showed lack of appetite, solitary, pronounced body color deformation and died after that.

**Key words:** deformity, growth, mortality Nile Tilapia, Vitamin B<sub>6</sub>, yeast.

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### **Stomach Contents of Green Mussels, *Perna viridis* (Linnaeus, 1758) in Sriracha Bay, Chonburi Province**

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Stomach contents of green mussels (*Perna viridis*) at Sriracha Bay, Chonburi province were studied

from November 2003 to March 2004, *Perna viridis* samples were collected twice a month. Stomach contents of a total of 200 samples were examined after collection. Phytoplankton, zooplankton and detritus were found. Phytoplankton was the main component. There were 24 genera of phytoplankton. They were composed of 18 genera of diatoms, 5 genera of dinoflagellates and 1 genus of silicoflagellate. The dominant phytoplankton in each month was *Bacteriastrum*, *Coscinodiscus*, *Cyclotella*, *Navicula*, *Nitzschia*, *Prorocentrum*, *Pseudonitzschia*, *Tabellaria* and *Thalassionema*. From this study, the highest phytoplankton was the first collection of November and the lowest phytoplankton was the second collection of March. They were 2,250 cells and 782 cells respectively. The diversity index of phytoplankton was highest in the second collection of January (3.35) and the lowest diversity index was in the second collection of November (3.00).

**Key words:** green mussel, *Perna viridis*, phytoplankton, stomach contents, Thailand.

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### **Laboratory Exercise for Teaching Photosynthetic Characteristics of Seaweeds in Science Classes in Japanese Junior High Schools**

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We developed a laboratory exercise to allow junior high school students to understand the relationships between the photosynthetic characteristics of seaweeds and their vertical distribution, which was reported on at the 18th AABE conference; the fronds of a red alga, *Mastocarpus yendoi*, and a green alga, *Ulva pertusa*, cultured under room conditions were used in the exercise. In the pre-

sent paper, we report both on the teaching plans, including the above-mentioned exercise as a wet-lab or a dry-lab, and on the implementation of these plans in ordinary and advanced junior high school science classes for Year 9 over a seven-year period to see if students can carry out the exercise within one school hour and how well students can understand the contents they learned through the exercise. In the advanced science classes, we asked students to measure the photosynthetic rate of either red alga or green alga under two different light colors, white light and green light. Within one school hour, students could complete the measurements of photosynthetic oxygen evolution from one of the algae under the different light conditions by means of the Productmeter. In ordinary science classes, students were given the raw data that were obtained both by the authors and by an advanced class and asked to draw graphs and analyze them. Most students could accomplish the task and could find that the red alga can perform its photosynthesis under green light as well as under white light while the green alga cannot. These results indicate that the teaching plans made are effective to allow junior high school students to understand the relationships between the photosynthetic characteristics of seaweeds and their vertical distribution.

**Key words:** junior high school, laboratory exercise, photosynthesis, seaweed, science class.

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### **How to Plant Forest**

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This paper reports on the recovery of biodiversity in experimental plots designed to test the frame-

work species method of forest restoration in Doi Suthep- Pui National Park, Northern Thailand. The framework species method involves planting up to 30 indigenous forest tree species, carefully selected for their ability to accelerate natural forest regeneration. Planted trees should restore forest structure and ecological functioning, whilst wildlife attracted by the planted trees accelerate biodiversity recovery through seed dispersal, resulting in recruitment of non-planted tree species. Bi-monthly inspections of framework species plots, ranging in age from 6 years to recently planted revealed that 41 of the planted tree species produced wildlife resources, such as flowers or fruits, within 7 years, with 14 doing so in less than 3 years. Birds' nests were recorded in 17 tree species within five years after planting. A total of 53 bird species were observed using the planted trees as perching sites and for feeding on fruits, nectar and insects. *Erythrina subumbrans* and *Melia toosendan* attracted the greatest diversity of bird species (28 and 24 species respectively). Planting 20-30 framework tree species in 10-rai plots each year, created a mosaic of different aged habitats which dramatically increased bird species richness at the landscape level, from about 30 species before planting began to 87 species at present. Of these, 45 species were also recorded in the nearest patch of surviving forest, representing about a 63% recovery in the total expected bird community within 6 years. Bird species most likely to disperse seeds of nearby forest trees into planted plots included Blue-throated Barbet, Black-crested Bulbul, Black-headed Bulbul, Flavescent Bulbul, Oriental White-eye and Japanese White-eye. Medium-sized mammals, which rapidly recolonized the plots included Hog Badger, Pangolin, Large Indian Civit, Barking Deer and Common Wild Pig. Live-trapping of rodents revealed the presence of *Mus pahari*, *Rattus bukit*, *R. exulans*, *R. koratensis*, *R. rattus* and *R. surifer*. All rodent species were also present in the non-planted control plots, except *R. exulans* and *R. koratensis*, which were

only recorded in planted plots 2-3 years old. Highest density of rodents was maintained in the non-planted control plots. Since these species are mostly seed-predators, a reduction in their population densities, following canopy closure in the planted plots, probably favours natural tree establishment. During a survey of naturally establishing trees, a total of 61 naturally establishing tree species (not including second generation seedlings of planted trees) were recorded in 6 year-old plots planted plots, compared with only 37 in control plots. Therefore, tree planting, and the seed-dispersing animals attracted by it nearly doubled the natural rate of tree species recruitment.

**Key words:** biodiversity, Doi Suthep- Pui National Park, forest restoration, framework species method, Thailand.

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### **Use of *Euglena* Beads (Immobilized *Euglena*) to Do Photosynthesis Exercises Easily**

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Algae are important organisms as producers in an aquatic ecosystem. Therefore, learning their photosynthesis through practical activities is important for secondary students to understand the overall features of aquatic ecosystems. Generally, the isolation and culture of microalgae need a lot of time and labor. In the case of *Euglena*, however, some simple culture methods have been developed. So, this alga is widely used in biology education. In the present study, to make the experiments on photosynthesis using a *Euglena* cell culture easier and more convenient, *Euglena* cells were immobi-

lized in calcium alginate gel beads (We refer to these beads as “*Euglena* beads”). After culturing the *Euglena* beads for about 20 days, they turned green and had a photosynthetic rate which was high enough to complete the measurement of photosynthetic rate with the Productmeter in a senior high school laboratory exercise within one school hour. Moreover, continuing to culture the *Euglena* beads resulted in higher photosynthetic rates. The *Euglena* beads were used instead of water plants in the qualitative experiment on photosynthesis which is commonly carried out in junior high school science classes in Japan. A pH indicator, BTB solution, was used for detecting photosynthetic CO<sub>2</sub> consumption in the experiment. About 300 *Euglena* beads, which looked dark green, were dipped in 20 ml of water containing the BTB solution and illuminated on an OHP. As a result, the color of the water changed from green to blue green in 30 minutes indicating that dissolved CO<sub>2</sub> was consumed. Therefore, it seems that the *Euglena* beads cultured for more than 20 days and appearing dark green can be used for quantitative and qualitative experiments on photosynthesis at both junior high school and senior high school levels.

**Key words:** *Euglena*, immobilization, high school science, laboratory exercise, photosynthesis.

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### **Three-Dimensional Structural Modeling of DNA Replication for A Study of Genetics**

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and Jintana Arunnopparat**  
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One of the problems in the classroom of teaching genetics is what students often find it hard to understand the lecturer’s explanation of genetics is-

sues. One reason is the use of two-dimensional media i.e. drawing and diagram which are unable to help students visualize genetic images. Therefore, we designed to test whether a three-dimensional model helps students’ learning. A three-dimensional model of DNA replication was invented. The model was constructed from materials i.e., plastic beads, plastic tubes, plastic plate, flannelette, and metal wire. Different colour beads represented deoxyribose sugar and nitrogenous bases, different segmented-colour tubes represented phosphate and hydrogen bonds, and flannelette represented RNA primer. They were all constituted to be a double helix DNA molecule with a “Y-shaped” replication fork. Each strand of the double helix at the fork acted as the template for synthesis of a leading and a lagging strand of new DNA molecules. This model was then tested with students in the Genetics classroom. The result in the classroom demonstration showed that three-dimensional models enhanced student learning of a complicated structure in the study of Genetics.

**Key words:** DNA replication, double helix, Genetics education, three-dimensional model.

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### **Biological Terms in Science Textbooks Used in Compulsory Education in Japan**

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The author has been researching effective ways of implementing biology education within compulsory education for many years. To serve as a basic reference for this work, an investigation into the usage of biological terminology in compulsory education science textbooks was performed. In Japan, Science is taught in the third and higher grades. The lowest grade covered in the investigation was necessarily the third grade, and as pupils at this level are still learning the language it-

self, the definition of “biological terms” used here includes not only technical terms for biology, but also words related to biology that are also used in daily conversation. As a result, “terms related to biology education” would be more precise than “biological terms.” The investigation includes the generation of a comprehensive list of related terms from all the approved, commercially-available science textbooks used in compulsory education, and notation of how many textbooks each of the terms is used in at each grade level (a maximum of six textbooks for elementary school or five for lower secondary school). This presentation outlines the results of that investigation.

**Key words:** biological terms, compulsory education, Japanese science textbooks, survey.

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### **The Development of An Outdoor Biology Education (OBE) Program Emphasizing Science Inquiry for Pre-service Elementary Teachers and Its Educational Effects**

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An outdoor biology education (OBE) program was developed for pre-service elementary teachers to improve their inquiry-based science teaching ability. A sample 160 students from a National University of Education in Korea participated the program and completed questionnaire about biology teaching efficacy. The OBE program was executed for 3 days and 2 nights at Upo Wetland, the largest wetland in Korea. The OBE program was done by groups of 3 to 4 students for safety and efficiency of inquiry. The students chose their theme and spe-

cific methods or procedures to explore Upo Wetland’s ecosystem. Then, they collected a variety of data, including samples of living things, measures, and photography, and analyzed the data, drew conclusions, and present the results. The scientific inquiry activities used in the program included basic and integrated process skills of SAPA (Science-A Process Approach). It was identified that the students’ self-efficacy on science and biology teaching were changed more positively after experiencing the OBE program.

**Key words:** education program development, outdoor biology, pre-service elementary teachers, science inquiry.

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### **The Use of Learning Resources for Teaching Biology**

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Education system in Thailand has recently changed. These changes necessitate revision of the national education curriculum which is a fundamental mechanism for the development of national education quality. The Ministry of Education (MOE) mandated changes specific to instructional process and announced the National Education Act (NEA) in 1999. The heart of the NEA is “all learners are regarded as being most important.” The NAE also includes an additional provision: “Lifelong education for all, continuous development of the bodies of knowledge and learning processes”

The government will promote the running and establishment, of all types of lifelong learning resources, namely: public libraries, museums, art galleries, zoological garden, public parks, botanical gardens, science and technology parks, sport and recreation centres, data bases, and other

sources of learning. The Institute for the Promotion of Teaching Science and Technology (IPST) has realized the change of educational system and is concerned with teachers and students who are facing these rapid changes. IPST has formed a network of teachers especially science and maths in 175 education areas throughout Thailand to stimulate interests in science through well-planned activities. The learning resources will support teachers for their inquiry teaching, designing activity sheet based on the environment of their local education area. Students will benefit by using these various sources of learning and finally they will be able to enhance the scientific skill from their investigation as well as to appreciate the value of natural environment which really needs sustainable preservations.

**Key words:** curriculum development, Institute for the Promotion of Teaching Science and Technology, lifelong education, National Education Act, Thailand.

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### **Development of Biotechnology Experimental Modules for Improving Creativity of the Science Gifted**

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The purpose of this study is to develop biotechnology experimental modules for improving creativity and achievement of the science gifted. The developed modules focused on learning cycle models that solve problems creatively in that the students lead to perceive problems rather than following the experimental process like a cookbook. The developed modules as a result of the study are

‘extracting DNA,’ ‘constructing paper DNA model,’ ‘electrophoresis of DNA,’ ‘handling of the restriction enzyme to DNA.’ They were applied to the biology classes at a high school for the science gifted, after dividing into the experimental group and the controlled group. The results showed that the students’ creativity and achievement of the experimental group which was applied the developed modules were more effective than the controlled group ( $p < 0.05$ ). The teachers and students who were experienced with the developed modules showed positive reflections to the questionnaire on the appropriateness and the effect of contents of the modules. This study means a great deal in the view of being able to instruct the biotechnological contents as designing the experimental modules that make students learn easily biotechnological concepts, improve their creativity and be provided for them to school science classes.

**Key words:** biotechnology, creativity improvement, experimental module development, high school.

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### **A Study of Preservice Elementary Teachers' Biology Teaching Efficacy Belief by Network Analysis through the Biology Outdoor Inquiry Work**

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This study explored the teaching efficacy belief of preservice elementary teachers on biology in outdoor inquiry work. Preservice teachers ( $N=68$ ) enrolled in biology outdoor inquiry course completed questionnaires to be analyzed for which area they were apt to be hard and apt to be easy to teach

on biology at the beginning and the end of the course, and analyzed for what they changed during the course at the end. Network analysis helps a qualitative data be analyzed more comprehensively and profoundly. Results revealed that, at the beginning of the course, many preservice elementary teachers had a thought that biological knowledge area was the most important, therefore they would try to have more biological knowledge, whereas at the end, some moved to science inquiry area, some to attitude area, but the portion of pointing out of knowledge as the most important thing for teaching biology had also remained high. Implications for research are discussed, and recommendations for educators and advisors are presented.

**Key words:** preservice elementary teachers, network analysis, outdoor inquiry course, teaching efficacy belief.

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### **Using “Avian Flu News” As An Inquiry Lesson Plan in the Secondary Biology Classroom**

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For a long period of time, Thai citizens are in panic over the outbreak of “Avian Flu” across Thailand. It is the IPST responsibility in promoting and bringing science to the classroom. Avian flu news is used as an inquiry lesson plan for the secondary biology classroom. In the quest to make students understand the viral outbreak situation, five steps of inquiry model incorporated in this lesson plan will intrigue student perception and understanding. The topic on cell context must be taught before starting this lesson. We will use it as background knowledge, link it to viral context, and infer it to news meaningfully. At the begin-

ning, we use the news headline as an **engagement step** to bring students into context we planned. Then, students will learn and compare cell and virus, answer questions / query in class, by using their background knowledge and the provided contents from teacher in **exploration step**. After they have learned and discussed, the provided contents are also used in **explanation step**. Next, from student resources about “Avian Flu Time Line,” “How to Protect Yourself from Avian Flu” and “Maps of world, Asia, and USA,” students will use these sources of information to analyze and find the pattern of outbreak, roughly, in **elaboration / extension step**. Finally, we use role-play and/or student poster to determine student’s outcome in **evaluation step**-the step that we find out what students have learned from this lesson. The aim of this pilot project is to create awareness and the application of the meaning of science context in the classroom to student’s daily life.

**Key words:** avian flu news, five steps model, inquiry lesson plan, secondary biology.

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### **Digital Photography in Microbiology Education: An Example from Actinomycete Research**

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For many years, the uses of 35 mm films either negative or slide have been a gold standard for photographic records in microbiological research and teaching. However, advances in computer technology have caused a paradigm shift, and digital presentations are quickly becoming standard for academic presentations. The rapid development of digital photography has made dra-

matic impact on the way of documentation and storage of biological information. The ease of use and its versatility in producing good quality images at affordable price make digital photography a convenient and preferable alternative to conventional 35 mm photography. In this presentation the basic principle of digital photography will be discussed, and the advantages and disadvantages of using digital photography and conventional photography also emphasized.

**Key words:** actinomycete, digital photography, microbiology education.

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### **A Survey of Medicinal Weeds Family Acanthaceae and Family Asteraceae in Chonburi, Thailand**

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A survey was conducted to categorize medicinal weeds family Acanthaceae and family Asteraceae found in Chonburi Province, Thailand. Places sampled on 12 areas in 4 districts were residential areas, agricultural lands, plantations, uninhabited areas, along the seashore and some aquatic habitats were also included. The result of this survey on family Acanthaceae had 7 genera and 11 species and family Asteraceae had 13 genera and 14 species. In total 25 species were cataloged belonging to 20 genera in 2 families. The medicinal characteristics of each weed species were studied in collaboration with traditional herb medicine providers as well as from historical manuscripts. Frequency of occurrence and medicinal characteristics of the weeds are discussed.

**Key words:** Acanthaceae, Asteraceae, medicinal weeds, survey, Thailand.

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### **Some Characteristics of A Potential Anti-microbial Producing Actinomycete Strain Isolated from Island Soil**

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Actinomycetes are gram-positive bacteria found mainly in soil which capable produce many of bioactive compounds especially antibiotics. The actinomycetes antibiotics are not restricted to antibacterial antibiotics but include antifungal, anticancer and antiviral antibiotics. An isolate of actinomycete strain SM17-1 isolated from Samaesarn island, Chantaburi was identified as *Streptomyces* sp. from morphological of hook, loop and spiral spore chains and chemical analysis of diaminopimelic acid of cell wall and sugar pattern of whole-cell hydrolysate studies which had L-DAP and had no characteristic sugar, respectively. The strain showed potent anti-microbial activity against both gram-positive bacteria and fungi: *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Pichia dispersa*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii* and weak inhibition activity of *Aspergillus niger*. For the biochemical tests: nitrate was reduced, urease positive, esculin, and gelatin were degraded, but glucose was not fermented. Arabinose, D-mannose, D-mannitol, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenyl acetate could not be utilized, but glucose and *N*-acetylglucosamine were utilized. Colonies on ISP2 medium were gray and the reverse was dark red. The strain is one of promising *Streptomyces* spp. for newly anti-MRSA substances.

**Key words:** actinomycete, anti-microbial production, island soil, *Streptomyces*.

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### **Peroxidase Production by Hairy Root Cultures of Some Vegetables**

**Vilaporn Boonyakitjinda and Vimol Kwankua**

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*Agrobacterium rhizogenes* strains 15834, 8196, 2659, A4, TISTR 510, and TISTR 511 were tested for ability to induce hairy roots on Chinese radish (*Raphanus sativus* L.), choysum (*Brassica chinensis* L.) and Chinese amaranth (*Amaranthus tricolor* L.). Surface-sterilized cotyledons and stems from sterile plantlets were immersed in a suspension of *A. rhizogenes* harboring pRi for 5, 10, 15 min and 1, 2 hr. in the dark and then transferred to a solid Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose and 0.5 mg/ml claforan, cultured at 25°C under 16 h light (3000 lux). In all plants and by all strains of *A. rhizogenes*, the hairy roots emerging from the leaf explants showed more vigorous growth and extensive lateral branches in the absence of phytohormones than those from the stems. Hairy roots of Chinese radish grew fastest. Hairy roots from leaf explants were excised and transferred to hormone-free liquid MS medium without claforan in complete darkness on a gyratory shaker. The growth of hairy roots was recorded after 35 days. Different strains of *A. rhizogenes* performed differently in the three cultures. TISTR 510, with the co-culture time of 15 minutes, caused the highest increase of hairy roots by having weight increased 3.07 times of its initial weight which was 3 times of the control in Chinese radish. However, strain 2659 did the best in choysum and Chinese amaranth. The co-culture time in choysum was 2 hours and the weight increased 7.05 and 3.75 times

of its initial weight and the control, respectively. For Chinese amaranth, the 5-minute co-culture time and 5.02 and 4.44 times of weight increase were recorded. Peroxidase activities (units/0.5 g fresh weight) were measured by spectrophotometer and were compared to the non-transformed controls. The highest increases of activities were 1.75, 1.32 and 1.48 times by strains 8196, 2659 and A4 in Chinese radish, choysum and Chinese amaranth, respectively. It is suggested that the productivity of peroxidase could probably be enhanced by selecting the appropriate hairy root lines and by improving the culture medium composition.

**Key words:** *Agrobacterium rhizogenes*, Chinese amaranth, Chinese radish, choysum, hairy root, peroxidase, transformation.

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### **Time Course of Changes in Blood Ammonia and Ammonia-N Excretion Rate in the Blue Swimming Crab, *Portunus pelagicus* L. Subjected to Different Salinity Levels**

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Changes in blood ammonia and ammonia-N excretion rate of *P. pelagicus* were determined in salinity levels of 25, 50, 75 and 100% SW after 0, 0.5, 1, 3, 6, 12, 24, 48 and 96 hr exposure time. It was found that concentrations of blood ammonia in different salinities were positively increased in relation to time. At 96 hr, the cumulative amounts of blood ammonia increased directly with salinity. In 25% SW, blood ammonia increased rapidly after 1 hr and the concentration is highest at 12 hr exposure, then mortality occurred. Ammonia-N excretion rates increased with decreasing

salinities. The highest and lowest ammonia-N excretion rates were  $26.08 \mu\text{g g}^{-1} \text{h}^{-1}$  in 25% SW and  $7.79 \mu\text{g g}^{-1} \text{h}^{-1}$  in 100% SW, respectively.

**Key words:** ammonia-N excretion, blood ammonia, blue swimming crab, *Portunus pelagicus*, salinity.

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### **Cryopreservation of Some Wild Orchid Seeds**

**Vilaporn Boonyakitjinda and Vimol Kwankua**  
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Seeds of four species of wild orchids, *Aerides falcata* Lindl., *Dendrobium cariniferum* Rchb.f., *Dendrobium heterocarpum* Lindl. and *Rhyncostylis retusa* (L.) Blume, were preserved by dehydration technique using laminar air flow and silica gel prior to storage in liquid nitrogen at  $-196^\circ\text{C}$ . The dehydration periods were 30 minutes and 1, 2, 3 and 10 hours for the laminar air flow whereas 30 minutes and 1, 2, 3 and 25 hours were varied for 50 g silica gel. The seeds were then stored in liquid nitrogen for 24 hours before being thawed in water bath at  $25^\circ\text{C}$ . For survival tests, they were cultured on a solidified half-strength Vacin and Went medium at  $25^\circ\text{C}$  under the 16-hr photoperiod. It was found that the survival rate (%) depended on the dehydration period as well as the orchid species. All seeds, which were stored in liquid nitrogen without dehydration, did not survive when they were cultivated later. The dehydration periods of 1, 2 and 3 hours were considered appropriate for both techniques since the % survival of *A. falcata*, *D. cariniferum* and *D. heterocarpum* seeds ranged from 50% to 100%. However, this was not the case of *R. retusa* of which the survival rate was highest at 50% after being dehydrated for 10 hours

by laminar airflow or 25 hours by silica gel. It should be noted that all viable seeds developed into green protocorms within one month, all of which developed into green shoots.

**Key words:** cryopreservation, seed germination, seed survival, wild orchid.

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### **Culture of *Aspergillus* on Wheat Bran and Enzyme Experiments – Analysis of Produced $\alpha$ -Amylases –**

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and Motoo ARAI<sup>2</sup>**

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Three species of *Aspergillus* (*Aspergillus oryzae*, *A. niger* and *A. sojae*) were cultivated on the wheat bran and  $\alpha$ -amylases were extracted from the culture. The enzymes were precipitated with ethyl alcohol and analyzed by zymography on native-PAGE. The  $\alpha$ -amylase activity bands were detected clearly on the gel by an iodine-starch reaction. The enzymes were immobilized in a calcium alginate gel sheet, and the effects of temperature on its starch-liquefying activity with iodine-starch reagent were studied. The methods of cultivation on the wheat bran and precipitation of enzymes with ethyl alcohol do not require any expensive tool. It is important for students to cultivate microbes, prepare some enzymes and analyze their enzymatic characteristics by themselves. Through these experiments, students increase their interest in experimenting on enzymes and learning the relationship between microbes and human life. So, a series of such experiments on enzymes are suitable as a teaching especially for inquiry and problem-solving activities in high school biology.

**Key words:** *Aspergillus*, culture, enzyme experiment, senior high school, inquiry and prob-

lem-solving activities, wheat bran.

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### Studies on the Shell Morphology of the Freshwater Snail *Paludomus Swainson*, 1840 in the Kwaie Noi and Kwaie Yai River Watershed, Kanchanaburi, Thailand

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The freshwater snail of *Paludomus Swainson*, 1840 collected from ten locations in the Kwaie Noi river watershed and five locations in the Kwaie Yai river watershed, Kanchanaburi, Thailand, was studied. These *Paludomus* freshwater snails are distinguished by shape, size, shell color pattern, suture, body whorl sculpture, operculum and the other basal periphery. All of them are dextral and the operculum is concentric with spiral. The shells are elongated and subglobose in shape. The surface is smooth and covered with a brownish or greenish periderm. Most of them show pointed apex, deep suture, and narrow columella. However, the snails of Tao Dum stream, one of the ten survey locations in the Kwaie Noi river watershed, are markedly different from the others in that they show eroded apex, shallow suture, and wide columella. In addition, when the ratios of the length of body whorl / shell length and size index are analyzed using ANOVA tests, Tao Dum snails exhibits significant difference ( $P < 0.05$ ) in the size index. Duncan multiple range test and Scheffe

test further support the present findings.

This work was supported by the Research and Development Institute, Silpakorn University, Thailand and the TRF/BIOTEC Special Program for Biodiversity Research and Training grant BRT T\_145038.

**Key words:** freshwater snail, *Paludomus*, shell morphology, survey, Thailand.

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### Cytogenetic Studies in Sanoe (*Sesbania* spp.)

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Cytogenetics studies have been undertaken in three species of Sanoe. They are *Sesbania javanica*, *Sesbania speciosa* and *Sesbania rostrata*. Root tip cells of *Sesbania* spp. were observed for karyotype pattern. In this study, Feulgen-stained squash method was the technique used for material preparation. The samples of primary root meristems of *Sesbania* were taken during 9.00-12.00 am. Two kinds of pretreatment agents, 0.01%-0.10% colchicines and 0.002-0.006 M 8-hydroxyquinoline, were used at 10-20°C for 1-5 hours. The result of the cytological procedure specifically suited to *Sesbania* spp. is shown in the table below.

**Key words:** cytology, Feulgen-staining, karyotype analysis, sanoe, *Sesbania*

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<i>Sesbania</i> spp.	Suitable time to take the sample	Pretreatment condition	Chromosome number	Karyotype pattern
Sanoe kin dok <i>Sesbania javanica</i>	9.30 am	0.004 M 8-hydroxy quinoline, 10-12°C, 4 hr	2n = 2X = 12	$L_2^m + L_2^{sm} + M_2^m + M_4^{sm} + S_2^{sm}$
Sanoe India <i>Sesbania speciosa</i>	9.15 am	0.002 M 8-hydroxy quinoline, 10-12°C, 3 hr	2n = 2X = 12	$L_2^{sm} + M_4^m + M_4^{sm} + S_2^m$
Sanoe African <i>Sesbania rostrata</i>	9.30 am	0.002 M 8-hydroxy quinoline, 10-12°C, 2-3 hr	2n = 2X = 12	$L_2^{sm} + L_2^m + M_2^{sm} + M_2^m + S_4^m$

## Primarily Study of Boletes Diversity in Doi Suthep-Pui National Park, Chiang Mai Province

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A survey on the diversity of boletes in Doi Suthep-Pui National Park was conducted during June 2003-July 2004. The average temperature and humidity during that time was between 18-27°C and 78-100%, respectively. Sixty specimens of boletes were collected, examined and identified by using keys of Corner (1972), Ellis and Ellis (1990) and Moser (1983). Boletes were most abundant between June to October during which temperature was 16-27°C, relative humidity was 82% and pH 4-6 in soil. At the same period in 1997-1998, boletes were found belonging to two families, *Boletaceae* and *Strobilomycetaceae*. In family *Boletaceae* there were *Boletus coccineinus*, *B. fragrans*, *B. impolitus*, *B. magnificans*, *B. queletii*, *B. radicans*, *B. regius*, *B. retisporus*, *B. speciosus*, *Gyroporus castaneus*, *Leccinum crocipodum*, *L. griseum*, *L. oxydabile*, *L. scabrum*, *L. thalassinum*, *Pulveroboletus ravelii*, *Rubinoboletus ballouii*, *Suillus granulatus*, *S. grevillei*, *S. placidus*, *Tyloporus humilis*, *T. nigerlimus*, *Xerocomus moravicus* and *X. rubellus*. In the family *Strobilomycetaceae*, there were *Boletellus emodensis*, *Porphyrellus porphyrosporus*, *Strobilomyces mollis* and *S. strobilaceus*. In this investigation the boletes species were found less than the previous reports. Eighteen species of boletes belong to 7 genera in the family *Boletaceae* and three species are from 3 genera of the family *Strobilomycetaceae* were found. Phylogenetic relationship using ribosomal DNA sequences (rDNA) within the interested taxa of boletes will be analyses in future studies.

**Key words:** *Boletaceae*, boletes diversity, Doi Suthep-Pui National Park, *Strobilomycetaceae*,

survey, Thailand.

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## Wild Mushrooms Database of Chiang Mai Community Forests

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The diversity of macrofungi at Chiang Mai community forests and Doi Suthep-Pui National Park during October 1996 – November 1998 and November 2003 – October 2004 was investigated. The fungi were collected from 19 forest areas and identified. Two hundred and fifty eight species were found. They were ascomycetes: 30 species, 21 genera, 12 families, 5 orders, and basidiomycetes: 228 species, 89 genera, 35 families, 10 orders. The most prominent species of macrofungi found belonged to the families *Boletaceae*, *Agaricaceae* and *Russulaceae* comprising 43, 35 and 28 species respectively. Wild mushrooms database was developed by using the data related to the environmental habitat, macroscopic and microscopic characteristics of wild mushrooms for creating the relating database and database management program (Wild Mushroom Database Version 1.0). This software is used for managing the dried wild mushrooms in the mushroom herbarium and it is more convenient in searching and learning.

**Key words:** Chiang Mai community forests, Doi Suthep-Pui National Park, mushroom database, Thailand.

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## Optimization of Chitinase Production by *Streptomyces* MK6-16

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Chitinase production by *Streptomyces* sp. MK6-16 was optimized by assessing activity over a range of culture conditions. Synthesis of extracellular chitinase was induced by chitin. Highest levels of enzyme activity were 1-29 units/ml, and a specific activity at 3.59 units/mg protein produced in the medium comprised of 1.5% ball-milled chitin, 3% wheat bran, 0.2% MgSO<sub>4</sub> 7H<sub>2</sub>O, 0.1% KH<sub>2</sub>PO<sub>4</sub>, 0.01% FeSO<sub>4</sub> and initial pH 6, using 3.85% (v/v) seed inoculum. Cultures were incubated at 26- 29°C with shaking at 200 rpm for four days. Optimum pH and temperature for chitinase activity were 5.0 and at 40°C, respectively. The enzyme hydrolysed colloidal chitin, ball-milled chitin, *Shizophyllum commune* cell wall and carboxymethyl cellulose but did not hydrolyse maltose, cellobiose and p-nitrophenol-β-D-N-acetylglucosamine. After precipitation with 40% saturation ammonium sulfate, the specific activity of chitinase increased about 2-fold compared with crude enzyme.

**Key words:** chitinase production, enzyme characteristics, optimization, *Streptomyces*.

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## A Preliminary Study on the Effects of Hydrocooling on Quality of Fresh Longan Fruit cv. Daw

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and Uraporn Sardsud<sup>2</sup>

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One-day old fresh longan fruit (*Dimocarpus longan* Lour. cv Daw) was used in our preliminary

study. The fruits were placed in 3 kg perforated plastic baskets of commercial scale for each treatment. The treatments were: no pre-cooling; tap water immersion; pre-cooled with iced water plus no chlorine, with iced water plus 50 or 100 or 200ppm chlorine respectively; for cooling time of 10 min until their final temperatures of longan fruit reached 5°C to determine the effects of hydrocooling treatments on quality and decay during storage at 5°C and 93% RH. After three week storage the results indicated that iced water plus 50ppm chlorine longan fruit treatment significantly decreased peel browning and decreased fruit decay in comparison with no pre-cooling and tap water immersion (the control). Pre-cooling plus 50 ppm chlorine which had maintained the highest L\* value both inner and outer peel, prevented peel desiccation, maintained both total soluble solids (TSS) in flesh, sensory evaluation and extended storage life of longan fruit. Higher chlorine concentration: 100 or 200ppm increased peel browning and fruit decay. Pre-cooling with no chlorine did not prevent fruit decay from fungi. Weight loss was not significantly affected by all treatments.

**Key words:** fruit quality, hydrocooling, longan fruit, postharvest treatment.

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## Molecular Systematics of the Magnaporthaceae and Allied Genera Based on Cladistic Analyses of Partial SSU and LSU rDNA Gene Sequence

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The phylogeny of the Magnaporthaceae and allied genera were investigated using individual and combined analyses of 18S and 28S nuclear ribosomal DNA (rDNA) gene fragments. DNA from 18 taxa representing 6 genera from Magnaporthaceae were amplified and sequenced. Additional sequences in this family from Gen-Bank were included in analysis. The data set (totally comprises 65 taxa) was analyzed phylogenetically using Bayesian and maximum parsimony. The Magnaporthaceae form a monophyletic order containing six major anamorph/ teleomorph genera, with 100% posterior probability in the Bayesian analyses and 93% maximum parsimony bootstrap support. Members of Magnaporthaceae were found to constitute a monophyletic lineage and seem closely related with other taxa in Diaporthales, Ophiostomatales, and Sordariales. The phylogenetic relationships of fungi in this study were compared and discussed based on three data sets of 74 taxa of SSU, 65 taxa of SSU and 58 taxa of LSU.

**Key words:** cladistic analyses, Magnaporthaceae, molecular systematics, phylogeny, rDNA sequence.

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### **Microscopic Observation and Pathogenicity Determination of Common Molds on Postharvested Longan Fruit cv. Daw**

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Longan (*Dimocarpus longan* Lour. cv. Daw) is among one of the important exported fruits in Thailand. This crop confronting with a severe

postharvest fungal rot disease problem. Study on the surface appearance using stereo microscopy showed that the fruit skin rough and uneven. Under scanning electron microscope, the surface of longan fruit consisted of scale and epidermal hair, in some areas the remnant of cuticle could be observed. The filamentous fungi were observed from fruit surface. Many genera of fungi were isolated from healthy fruit skin of harvested longans by tissue transplanting method, such as, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium Pestalotiopsis*, *Phomopsis*, *Rhizopus*, *Trichoderma*, *Verticillium* and 7 genera unidentified. Each of the fungal isolate was inoculated onto the pericarp of the fruit for pathogenicity determination. Pathogenic ability showed that the common mold found on the fruit skin, *Lasiodiplodia* and *Pestalotiopsis* caused the most severe symptoms and rot the fruit rapidly, respectively. The most virulent isolate was identified as *Lasiodiplodia theobromae* based on morphological characteristics and DNA sequencing.

**Key words:** common molds, longan fruit, microscopic observation, pathogenicity.

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### **Effect of Some Edible Mushroom Extracts on Fruiting Body Formation of *Volvariella volvacea***

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Young fruiting bodies of some edible mushrooms i.e., *Pleurotus ostreatus*, *Lentinula edodes* and *Agrocybe cylindracea* were homogenized sepa-

rately with distilled water, methanol and acetone (500g/l). The aqueous extract was filtered through clean cheesecloth whereas the acetone and methanol extracts were concentrated by evaporation using rotary evaporator. The extracts were diluted to 1:10 with distilled water and added into potato dextrose agar (PDA) before sterilization. The plate agar medium was inoculated with mycelial plug of straw mushroom (*Volvariella volvacea*) obtained from the growing edge of 5 to 7-day old colony on PDA, and incubated at  $30\pm 2^{\circ}\text{C}$ . During incubation, the growth of the colony on PDA was measured everyday for 7 days. The results revealed that the aqueous extract of *P. ostreatus* gave faster growth of the straw mushroom than that of the control group and those grown on the other extract incorporated media. Two fold serial dilutions of the aqueous extract from *P. ostreatus* were sprayed on the surface of moist cotton waste in plastic bags fully colonized with straw mushroom mycelia (12-day old). After 5 days of incubation at  $30\pm 2^{\circ}\text{C}$ , it was found that the extract: water (1:1) significantly stimulated fruiting body formation.

**Key words:** edible mushroom extract, fruiting body formation, *Pleurotus ostreatus*, straw mushroom, *Volvariella volvacea*.

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<Country Reports>

### **Current Situation of the Japanese Society of Environmental Education**

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The Japanese Society of Environmental Education (JSEE) was established in May 1990 and we celebrated its 10th anniversary in 2000. The object of this report is to give an overview and describe the

present activities and perspectives of the JSEE. At the present time, the Law Concerning the Encouragement of Willingness for Environmental Conservation and the Promotion of Environmental Education (Law No.130) has been in force since October 1, 2004 and a UN Decade of Education for Sustainable Development (UN DESD) will be initiated by the United Nations from 2005, so it appears of interest to introduce a brief history, as well as current activities and perspectives of the JSEE.

#### **Overview - A Brief History of the JSSE -**

Environmental education (EE) in Japan has originated from unfortunate pollution problems such as Minamata disease, Yokkaichi asthma, Itai-itai disease or Agano Minamata disease (2nd Minamata disease). We called the EE during the period of high economic growth in the 1960s the pollution education or the nature-protection education. In the latter half of the 1980s as the threat of environmental problems was becoming globally acknowledged and conservation issues were attracting more attention, the importance of EE became increasingly recognized by foresighted educators, researchers and policy makers. In response to this heightening environmental awareness, the JSSE, an academic organization, was founded in 1990. In corporation with other educational associations founded at that time and with many educational NPOs/NGOs, the JSEE helped in many kinds of EE programs and projects in Japan. These initiatives enabled EE to branch out in many directions. A diversity of educational activities was established during the 1990s. The Ministry of Education, Science, Sports and Culture (MEXT) established a guideline for EE in 1991 leading to the introduction of EE in schools throughout the country. EE is now becoming more common in schools, and environmental in-service training programs for teachers have begun. Information exchange networks connecting schools have been enhanced and expanded. At teacher training college research centers for EE have been created

which are examining interdisciplinary ideas and theories in cooperation with the society. The Basic Environment Law, enacted in 1993, explicitly endorses the promotion of EE. Public awareness of the importance of conservation along with administrative support helped the increasing expansion of EE in the 1990s. More and more local governments have developed environmental master plans, launched environmental learning centers, established training programs for future leaders and provided learning opportunities for the public. Now, we would like to introduce “Environmental education: a critical challenge for the 21<sup>st</sup> century,” “Goals of Environmental education” and “Aims of the JSEE” by referring to the brochure of the JSEE (March, 2002).

**Present Activities of the JSEE**

*The number of members of the JSEE*

As a whole, during the 15-year period since 1995, the number of the members has a tendency to move toward increase.

*Financial situation of the JSEE*

At the present time, the membership fee is as follows:

Membership categories	Annual fee (yen)*
Regular member (Individual)	
Professional	5,000 (46.00)
Student	3,000 (28.00)
Group member (Corporate/Institutional)	10,000 (92.00)
Supporting member (sponsoring organization)	20,000 (184.00)

\*US\$ in parenthesis. US\$ 1.00 = ¥ 109.00 (Oct. 20, 2004)

**Key Words:** Japanese Society of Environmental Education.

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**Development of Education Program on Biology Research Ethics in Korea: A Short Report**

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It has become more and more appreciated in South Korea that appropriate research integrity (encompassing not just moral sensitivity but also methodological, social and legal sensitivity) is crucially needed in biology research, especially in the context of (potentially) controversial biotech-related topics. This appreciation reflects into the fact that the Korea Science Foundation is now funding an interdisciplinary, three-year research of which main goals are developing and implementing a research ethics course for biology-major students (particularly at the graduate-level). Four scholars with complementary expertise (molecular biology, philosophy of science, bioethics, ethics education) are participating in the research, and the research is now in the middle of its first-year.

The report explains the aims and objectives of this research, and introduces the core ideas of its first task, the internet-survey on research integrity of Korean biology researchers. Some preliminary results from the survey will be presented and discussed.

**Key words:** biology research ethics, education program development, graduate level, Korea.

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