INTERNATIONAL CONFERENCE ON BIOLOGICAL SCIENCE

ADVANCES IN BIOLOGICAL SCIENCE:
Respect to Biodiversity from Molecular to Ecosystem
for Better Human Prosperity

PROCEEDINGS

Organized By

Faculty of Biology Universitas Gadjah Mada
Yogyakarta, Indonesia
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PREFACE

This publication reports papers presented at the International Conference on Biological Science Faculty of Biology Universitas Gadjah Mada 2009 (ICBS BIO-UGM 2009), Advances in Biological Science: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity, organized by and held at the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia on October 16-17, 2009. The conference addressed a range of important research from various fields in biological science likely to play role in the improvement of human prosperity. Three kinds of session were held at the conference: plenary session featuring keynote and invited papers, oral presentation session, and poster presentation session. This proceeding features a number of papers presented in these sessions, which represent 5 themes covered in the conference, i.e. genetics and molecular biology, ecology and conservation, systematics and evolution, physiology and developmental biology, and biomedics.

I wish to thank my fellow Organizing Committee for their efforts towards the success of the conference. On behalf of the Organizing Committee, I wish also to thank keynote speaker, all invited speakers, paper presenters, academic reviewers, participants, and sponsors who have made the conference a success. Last but not least, I hope that the conference leaves us and all participants with memorable and fruitful experience.

Maryani
Chair of the Organizing Committee
WELCOMING SPEECH FROM CHAIR PERSON OF THE ORGANIZING COMMITTEE

Distinguish guests

- Rector Universitas Gadjah Mada, Prof. Ir. Sudjarwadi, M.Eng., Ph.D
- Keynote Speaker, invited speakers, participants, sponsorship, ladies and gentlemen

Good morning and may God be with us

It is my great privilege to greet you all to the International Conference on Biological Science Faculty of Biology Universitas Gadjah Mada 2009 (ICBS BIO-UGM 2009), Advances in Biological Science: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity, held in Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. I realize that you are all fully dedicated to the sessions that will follow, but I do hope that you all will also take time to enjoy our fascinating Yogyakarta, with its education trade mark, city of culture, batik, as well as its multicultural people.

Ladies and gentlemen, I recognize that this conference is principally designed to enhance the contribution of biological science to the development of other applied sciences related towards a more sustainable use of biological resources. With this fast development of studies and researches on biological topics, we realize that biology highly contribute to applied sciences and sectors, including medicine, pharmacy, agriculture, veterinary, and food as well as health industries. In this case, I am very alert to the increasing needs to understand biology in respect to biodiversity from molecular to ecosystem beneficial in the improvements of human prosperity. Therefore, I wish that this event will be a great opportunity and a wonderful venue for us to lay down a cooperative framework and to establish scientific collaboration between scientists internationally. An impressive roster of distinguish speakers and attendants from Japan, Malaysia, Egypt, and Indonesia has been gathered in this conference.

Hereby, on behalf of the Organizing Committee, I acknowledge Prof. Dra. Sukarti Moeljopawiro, M.App. Sc., Ph.D. (Universitas Gadjah Mada) as a keynote speaker, and also to these following invited speakers, Prof. Dr. Yasunori Machida (Nagoya University, Japan), Chiyoko Machida, Ph.D. (Chubu University, Japan), Prof. Dr. Hitoshi Sakakibara (RIKEN Plant Science Center, Japan), Prof. Ir. Bambang Sugiharto, M.Agr.Sc., Ph.D. (Universitas Jember, Indonesia), Prof. Dr. Campbell O.Webb (Harvard University, USA), and Dr. Richard Noske (Charles Darwin University, Australia), for delivering their valuable scientific information.
To make this program happen, I would like to gratefully acknowledge to the valuable contributions from personal and institutional sponsorship and funding including Institute for Research and Community Services Universitas Gadjah Mada, PT. Fajar Mas Murni Semarang, ILLUMINA PT. Pandu Anugerah Analitika, Drs. H. Maryadi Broto Suwandi, M.Kes Yogyakarta, Prima Grafika Yogyakarta, Argus Optical Yogyakarta, and BTKL (Balai Teknik Kesehatan Lingkungan) Yogyakarta.

I also gratefully thank to the Dean and Vices Dean of Biology Faculty, Universitas Gadjah Mada for giving us opportunity and support to organize this conference. Heartfelt thank is delivered to the Steering Committee, the Academic Reviewers, members of the Organizing Committee for their strong support, active participation, cooperation and hard works throughout this year in preparing and organizing this meaningful meeting and to those who have contributed their untiring effort in making this conference success.

Despite our best efforts, it is inevitable that there is a lack in organizing this conference and I profoundly apologize to all invited speakers, oral and poster presenters, attendants, donators and committee members.

Finally, I would like to offer my best wishes for a highly enjoyable, successful, productive and fruitful conference.

Thank you

Maryani
Chair person of the Organizing Committee
OPENING REMARKS FROM THE DEAN OF FACULTY OF BIOLOGY UGM

Distinguish guests,

- Rector Universitas Gadjah Mada, Prof. Ir. Sudjarwadi, M.Eng., Ph.D.
- keynote speaker, invited speakers, and dear participants,

Assalamualaikum Wr.Wb. May God give us health and happiness

Welcome to Yogyakarta, the city of youth, education, and culture. It has been an honors for me to be here standing in front of you to speak in the prestigious International Conference on Biological Science, Faculty of Biology Universitas Gadjah Mada (ICBS BIO-UGM) 2009 with special theme of “ADVANCES IN BIOLOGICAL SCIENCE: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity”, that invited 9 honorable speakers mostly from foreign countries including Japan, Australia, United States of America. My special gratitude to the speakers who have spent their time travelling to Indonesia in your busy activity. This international conference also attracts more than 200 scholars and students mostly come from Indonesia, and some participants come from Egypt, Japan, and Malaysia. This occasion is such a good opportunity for us to share our experiences in research and good practices done that could inspire students and other researchers. Furthermore, it is also a chance for creating research collaboration among participants.

In line with vission of the Faculty of Biology UGM that the institution will be one of the center of excellence for higher education nationally that generate biologists who respect to tropical biodiversity researches. Therefore, national and international conferences will be held regularly in order to support local researchers and students in mastering their research communication competencies. New paradigm of Education for All (EFA) born by UNESCO nowadays is focusing on Education for Sustainable Development (EfSD). It promotes quality of education based on values, principles, and practices necessary to respond effectively to current and future challenges that includes all people. My deep appreciation goes to the Steering Committee, Academic Reviewers and the Organizing Committee that spend almost their valuable time to review articles and also to manage and organize this conference effectively. I also acknowledge our sponsors either institutional or individual, without their contribution this conference may not happen.
I wish this two days conference will enlighten tropical biological researches and researchers in Indonesia and give benefit to all of us. Thank you

Yogyakarta, October 16th, 2009

Dr. Retno Peni Sancayaningsih, M.Sc.
OPENING REMARKS FROM THE RECTOR UNIVERSITAS GADJAH MADA

Distinguished guests, ladies and gentlemen

On behalf of the Gadjah Mada University, I wish to congratulate and express my gratitude to the Faculty of Biology UGM and to the Organizing Committee of the International Conference on Biological Science (ICBS) 2009: Advances in Biological Science: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity for succeeding this conference. My sincere thanks are also addressed keynote speaker and all invited speakers to support this conference.

Biology is a core of fundamental science and the contribution of applied biology sector on the national economic development for Indonesia needs to be strengthened through the effort of developing prospective domestic and export of potential biodiversity and biotechnology products as mentioned in this conference theme. We still have some problems in biodiversity and biotechnology sector and that is why, this conference is now being conducted.

I wish, the meeting will be successfully bring the audience to exchange and brainstorm the scientific knowledge in order to provide valuable results for supporting the national biodiversity and biotechnology development. I also strongly hope that some ideas produced in this conference will be applied for practical application of biology in Indonesia in the near future.

Thank you and have a nice conference

Prof. Dr. Ir. Sujarwadi, M.Eng.
The Rector of Gadjah Mada University
Yogyakarta, Indonesia
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- Dr. Retno Peni Sancayaningsih, M.Sc (Dean Of The Faculty Of Biology, UGM, Indonesia)
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• Dra. Retna Widyastini
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• Harsana
• Dodo Priyatna
• Giyarto
• Harjono
• Darsono
• Nahrowi
ACKNOWLEDGMENT

The Following personal and Organization are gartefully acknowledged for Suporting this International Conference on Biological Science (ICBS 2009 BIO-UGM)
ADVANCES IN BIOLOGICAL SCIENCE:
Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity

Institute for Research and Community Services
Universitas Gadjah Mada, Yogyakarta, Indonesia
PT. Fajar Mas Murni Semarang, Indonesia
ILLUMINA PT. Pandu Anugerah Analitika, Indonesia
Drs. H. Maryadi Broto Suwandi, M.Kes Yogyakarta, Indonesia
Prima Grafika Yogyakarta, Indonesia
Argus Optical Yogyakarta, Indonesia

BTKL (Balai Teknik Kesehatan Lingkungan) Yogyakarta. Indonesia
BNI UGM Branch, Yogyakarta
Thematic Oral Presentation

Topic 1: Molecular Biology, Genetics and Bioinformatics

1. Leaky and Linear Scanning Translation of Chicken Anemia Virus Leader Sequence
   Afiono Agung Prasetyo, Toshio Kamahora and Seiji Kageyama

2. Efficient Translation Initiation Directed by Cap-dependent Chicken Anemia Virus Leader Sequence in Stress Condition
   Afiono Agung Prasetyo, Toshio Kamahora and Seiji Kageyama

3. Metagenomic Sequence Analysis Reveals Diverse 16S rRNA Gene In Termite Intestine Bacteria
   Irvan Faizal, Retno Lestari, Agung Heru Karsono, Abdul Latif, Dudi Hadianto, Nila Kusumawati, Indra Rachmawati, Bambang Marwoto, Nadirman Haska and Wahyu Purbowasito

4. Genomic and Proteomic Characterization of Insulin Receptor (hINSR) of DM Patients
   Fatchiyah, S. Widyarti, W. Fajriani, M.N.Y. Putri, L. Firdausi and DW. Soeatma

   Budi Setiadi Daryono

6. The Study of PIT1 Gene Polymorphism in the Najdi Cattle Using PCR-RFLP Method

7. Detection of Pib (Pyricularia oryzae resistance-b) Gene in Indonesian Local Rices (Oryza sativa L.)
   Annisah Isnaini and Budi Setiadi Daryono

8. Study of Sugarcane SucroseTransporter cDNA by Functional Expression in Yeast
   Slameto, Bambang Sugiharto, Kim Kyung Min and Ryza Aditya Priatama

9. Cloning and Transient Expression of Promoter from Elongation Factor 1 Alpha Gene (MeEF2) from Cassava (Manihot esculenta Carntz.)
   Sony Suhandono, Armelia Apriliandi Melkias, Lidya, Tati Kristianti and Nadia Hanum

10. Cloning and Sequence Analysis of Stearoyl-acyl Carrier Protein Desaturase (sad) Gene from Oil Palm (Elaeis guineensis Jacq.)
    Sony Suhandono and Dewi Yustika Sofia

11. In silico Docking and Molecular Dynamics Simulation of New Alkaloids from Rauvolfia caffra Stem Bark
    A. Pamuji, H. Sudrajat and U. Lathifah

12. Expression of Parthenocarpic Gene, DefH9-iaaM On Transgenic Tomato Lines
    Saptowo J.Pardal, R.Purnamaningsih, E. G.Lestari and Slamet

Topic 2: Ecology and Conservation

1. Density and Biomass of the Macrobenthic Fauna of the Intertidal Area in
1. Chloroplast DNA Sequences to Reveal Phylogenetic Relationship of Indonesia Banana Cultivars
   **Amin Retnoningsih and Y. Ulung Anggraito**

2. Genetic Diversity Analysis of Indigenous *Bacillus thuringiensis* Isolates Pathogenic to *Crocidoloma binotalis* by Using Molecular Phylogenetic Approach Based on 16S rRNA Gene Sequences
   **Langkah Sembiring, Christina L. Salaki, Jesmand Situmorang and Niken S.N. Handayani**

3. The Comparison of Morphological and Molecular Characters of *Vanda tricolor* Lindl. Merapi, Bali, East Java and West Java Forms
   **Endang Semiarti, Aziz Purwanto, Rindang Dwiyani, Tantri Swandari, and Esti Sri Lestari**

4. Invasion of Waterhyacinth (*Eichornia crassipes*) and Golden Apple Snail (*Pomacea canaliculata*) in Mangrove Ecosystem Segara Anakan, Central Java
   **Tjut Sugandawaty Djoohan**

5. Nutrient Input via Rainfall, Troughfall and Stemflow in the Low Montane Forest Gn. Gede Pangrango
   **Joeni S. Rahajoe and Alhamd L.**

6. Soil enzymatic activities of natural forest in permanent plot of low land national park “Gunung Gede Pangrango”
   **Antonius Sarjiya, Fauzi Rachmat, and Joeni Setijo Rahajoe**

7. Phyto Remediation Potential of *Salvinia molesta* and *Eichornia crassipes* in the Water that Contaminated by Sidoarjo Mudflow
   **Dewi Hidayati, Aunurohim, Irmina Kris Murwani and Atika Ayu Permatasari**

8. Effort for ex-situ conservation of Some Cultivars of Clove *Syzygium aromaticum* (L.) Merrill et. Perry and Its Rhizosphere Microorganism from Temate and Sapaaru, North Maluku
   **R.P. Sancayaningsih, A. Indriyanto and E.H. Poentyanti**

9. The Distribution Pattern and Abundance of Asteroid and Echinoid at Ringgung Waters South Lampung
   **Arwinsyah Arka, Agus Purwoko and Oktavia**

10. Reintroduction and Survivorship of the Threatened Palm *Pinaga Javana* Blume in the Gunung Halimun National Park, West Java
    **Didik Widyatmoko**

11. Low Biomass of Macrobenthic Faunat at a Tropical Mudflat: An effect of Latitude?
    **Agus Purwoko and Wim J. Wolff**

12. Toward The Indonesian Redlist Book: Species Priority Setting for Conservation of Indonesian Threatened Plants
    **Didik Widyatmoko and Rosniati A. Risna**

13. Chloroplast DNA Sequences to Reveal Phylogenetic Relationship of Indonesia Banana Cultivars
    **Amin Retnoningsih and Y. Ulung Anggraito**

14. Genetic Diversity Analysis of Indigenous *Bacillus thuringiensis* Isolates Pathogenic to *Crocidoloma binotalis* by Using Molecular Phylogenetic Approach Based on 16S rRNA Gene Sequences
    **Langkah Sembiring, Christina L. Salaki, Jesmand Situmorang and Niken S.N. Handayani**

15. The Comparison of Morphological and Molecular Characters of *Vanda tricolor* Lindl. Merapi, Bali, East Java and West Java Forms
    **Endang Semiarti, Aziz Purwanto, Rindang Dwiyani, Tantri Swandari, and Esti Sri Lestari**

**Sembilang National Park, South Sumatra, Indonesia**

**Agus Purwoko and Wim J. Wolff**
4. The Diversity of Foliose Lichens in the Forest of Tahura R Soeryo, Batu, East Java
   Miftahul Jannah, Dwi Anggorowati Rahayu, Devi Arifianti Mahadi, Murni Saptasari and Ludmilla Fitri Untari

5. A Preliminary Study of Moss Flora of Mount Lumaku, Sabah, Malaysia
   Fadzilah Awang Kanak, Monica Suleiman

6. Phylogenetic relationship of the Genus Trichotosia species in Sabah, Malaysia
   Norhaslinda Malekal and Monica Suleiman

   Rina Sri Kasiandari and Armesi Sugara

8. Morphological Variation of *Cibotium barometz* from West Sumatra
   Rugayah, Titien Ng. Praptosuwiryo and D.M. Pustaningtyas

9. The Distribution of West Malesian Fern Genus Diplazium (Woodsiaceae) Inside and Outside Malesia
   Titien Ngatinem Praptosuwiryo, Edi Guhardja and Dedy Darnaedi

10. Species Diversity of Rattans in the Genus Calamus in Muna Regency, Southeast Sulawesi, Indonesia
    Ratna Susandarini, Andilompo, Purnomo

11. Tuber Morphology Variation and Classification of Yogyakarta Water Yam (*Dioscorea alata* L.) Cultivars
    Purnomo and Ratna Susandarini

**Topic 4: Physiology and Developmental Biology**

1. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Increase Desmin Expression In Mouse Fetuses Palatal Tissue
   Salomo Hutahaean, S. Mangkoewidjojo, M. Sagi, dan W. Asmara

2. Dimethyl Sulfoxide’s Effect on Sperm Quality of Goramy Fish, *Osphronemus goramy* Lacepede, 1801 twenty four hours post-cryptopreservation
   Abinawanto, Mariana D, Bayu I and Ade Sunarma

3. Molecular Effects of Methoxyacetic Acid on Human Skin Fibroblast Cells in *vivo*
   Didik Priyandoko, Nashi Widodo, Tetsuro Ishii, Renu Wadhwa and Sunil C. Kaul

4. Stress Induced Microspore Embryogenesis in Mimulus
   Ari Indrianto

5. Mechanistic Studies Exploring the Effect of Absisic Acid on Gibberelin Contents
   K. Dewi and P.M. Chandler

6. The Effect of Storage Periode and Gibberellin on Dormancy Breakage and Amylase Activity of Rice Seeds (*Oryza sativa* L. var C64)
   K. Dewi and R.R. Aisyandari

7. The Growth of *Chlorella pyrenoidosa* in wastewater of molasses ethanol fermentation (vinase)
   Theresia Tri Suharni and Ludmilla Fitri Untari

8. How do Plants Respond to Nitrogen-shortage by Regulating Nitrate Uptake?
   Takatoshi Kiba and Hitoshi Sakakibara
9. Fungal Diversity Inside The Semuluh Cave, Semanu, Gunung Kidul, Yogyakarta
   Endah Retnaningrum, Anastasia Wahyu Widayati and Ratih Aryasari

10. Tiller Number Comparison among Three Hybrid Rice in Seedling Methods
    Setiarti Sukotjo

11. Classical Quantitative Structure-Activity Relationship Studies of Flavylum Salts as Xanthine Oxidase Inhibitors
    N.A. Iriani, H. Sudrajat and U. Lathifah

Topic 5: Biomedics

1. Extraction and Detection of Xylooligosaccharides: Introducing the Use of Prebiotic Cosmetics
   Anak Agung Istri Ratnadewi, Muhammad Naqib and Agung Budi Santoso

2. Molecular Genotyping of HBV by using Nested PCR and RFLP among Hepatitis B Patients in Yogyakarta and Surrounding Area
   Aris Haryanto, Nenny Sri Mulyani, Titis Widowati and Nastiti Wijayanti

3. Antioxidant properties of two genus of Zingiberaceae (Etingera spp. and Zingiber spp.) from Sabah, Malaysia
   Farrawati Sabli, Maryati Mohamed, Asmah Rahmat and Mhd Fadzelly Abu Bakar

4. Papuan DNA Mitochondrial Database, a Genomics Resource Supporting Population Genetics Studies and Biomedical Research
   Richardo Ubyaan, Irwandi Y. Suaka, Epiphani I. Y. Palit, Semuel Unwakoly and Yohanis Ngili

5. Detection and Identification of Influenza Viruses by Polymerase Chain Reaction (PCR)
   Akhirta Atikana, Ungke Anton Jaya and Herman Kosasis

6. Cloning of Large Hepatitis B Surface Antigens (L-HBsAg) from Indonesian Isolates : Development of the Third Generation Vaccine
   Dina Rachmi Ramdhani and Ernawati Arifin Giri Rachman

7. Essential Oil Profile of Temu Lawak (Curcuma xanthorrhiza Roxb.) Callus After Having Photoperiod and Sucrose Treatments
   L.Hartanto Nugroho, I.Sumardi, M.Wisnu and M.Peramanawati

8. Phytochemicals, Antioxidant and Anticancer Properties of Boesenbergia Species (Zingiberaceae) Endemic to Borneo
   Ling Jing Jing, Maryati Mohamed, Asmah Rahmat and Mohd Fadzelly Abu Bakar

9. Antifertility Activity of Flagellaria indica L. Fruit Extract on Female Albino Mice (mus musculus L.) Swiss Webster
   Yohanes E. Gunawan, Meda, G. SaraHayu, A. Haryono, and Suatma

10. Molecular Effects of Methoxyacetic Acid on Human Skin Fibroblast Cells in vitro
    Didik Priyandoko, Nashi Widodo, Tetsuro Ishii , Renu Wadhwa and Sunil C. Kaul

Rani Sasmita, Praparsiri Kanchanopas-Barnette and Kashane Chalermwat
## THEMATIC ORAL PRESENTATION SCHEDULE

**Topic 1: Molecular Biology, Genetics and Bioinformatics**

1. Development of an Efficient *Agrobacterium*-Mediated Transformation Method for Sugarcane  
   **Didik Pudji Restanto, Bambang Sugiharto and Kosum Ishiki**

2. Study on the Interaction between OsKANADI1 and a florigen Hd3a in Rice  
   **Yekti Asih Purwestri, Hiroyuki Tsuji and Ko Shimamoto**

3. Genetic Polymorphism of mt-DNA Cytochrom b (cyt b) in Indonesian Domestic Cattle  
   **Muhammad Cahyadi, Wayan T. Artama and Tety Hartatik**

4. Genetic Relationship between Ongole Hybrid Cattle and *Bos javanicus* in Indonesia based on Partial D-loop mtDNA  
   **Tety Hartatik, Tri Satya Mastuti Widi, Muhammad Cahyadi and Muhammad Affan Mu'in**

5. Diagnostic Sensitivity Comparison between Polymerase Chain Reaction-High Resolution Melting (PCR_HRM) and Sequencing Techniques to Detect KRAS Mutation  
   **Farid Sastra Negara**

6. Mutations Analysis in D-loop Region of Mitochondrial DNA in Human Gastric Tissues and Its Influence on the ROS and Cell Cycle  
   **Samuel Unwakoly, Felicia M. Lekatompessy, Epiphani I. Y. Palit, Richardo Ubyaan and Yohanis Ngili**

   **Tuti Arisuryanti, Agista Zaziroh and Dessy Nurul Astuti**

8. Chromosom Characterization of *Citrus nobilis* Lour, *Citrus microcarpa* Bunge, and *Citrus amblycarpa* (Hassk.) Ochse  
   **Tuti Arisuryanti, Herlianti Annisa, Idha Nur Chasanah and Gerhana Yuhan Rohyana**

9. The Inheritance of Random Amplified Polimorphic DNA (RAPD) Markers Linked to Powdery Mildey Resistance Gene in Melon  
   **Rina Sri Kasiamdari, Ganies Riza Aristya and Budi Setiadi Daryono**

10. Insertion of Activation Tag into *Batutegi* and *Kasalath* Rice Cultivar  
    **Vincentia Esti Windiastri, Eva Erdayani and Satya Nugroho**

11. A DNA Primer for Recognizing Porcine in Meat Products  
    **Tigor Nauli and Zalinar Udin**

**Topic 2: Ecology and Conservation**

1. Impact the Ciliwung River Environment on Its Water Quality  
   **Dwi Agustiyanı and Sarjiya Antonius**

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GENETIC POLYMORPHISM OF mt-DNA CYTOCHROME B (CYT B)
IN INDONESIAN DOMESTIC CATTLE

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ABSTRACT

The aim of this research was to identify nucleotide polymorphic sites in a 464 bp region of the cytochrome b (cyt b) mitochondrial gene of Indonesian Domestic Cattle (Bos indicus and Bos javanicus). This region is widely used as a target polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for species identification studies. We used four cattle breed as samples, there were Bali, Madura, Ongole-Grade (PO) and Local Pacitan cattle breeds. The L14735 and H15149 primer pair was used to amplify the cyt b gene. The PCR products were cleaved by TaqI restriction enzyme and then electrophoresed on 2% agarose gels. This research subjected to determine the diversity of domestic cattle base on maternal inheritance. The results showed that Indonesian domestic cattle had three haplotype. The domestic cattle which consist of Bali cattle, Madura cattle and Local cattle of Pacitan included in haplotype A, the PO cattle included in haplotype A and B, and one of Local cattle of Pacitan belong to haplotype C. The haplotype A indicated that the cattle related to Bos javanicus (Banteng), while Haplotype B and C indicated that the cattle related to Bos indicus. Indonesian domestic cattle had genetic diversity based on genetic polymorphism of mt-DNA cytochrome b.

Key words: PCR-RFLP, mtDNA cyt b, Indonesian Domestic Cattle, Haplotype

INTRODUCTION

The developing of domestic cattle is very important for Indonesian livestock industry. Huitema (1982) reported there domestic cattle has better reproductivity, more adaptable in tropical environment and management, and more resistant from tropical disease. Indonesian have four domestic cattle breed, such as Bali, Madura, PO and Local cattle of Pacitan (native Pacitan cattle).
To study the genetic polymorphism of domestic cattle in Indonesia, we examined the sequence of mt-DNA cytochrome b in several breeds which collected from the difference location. Mitochondrial DNA had been used as a molecular marker. Mitochondrial DNA evolves much faster than nuclear (nc) DNA and thus contains more sequence diversity compared to nuclear DNA, facilitating the identification of closely related species (Brown et al., 1996). In addition, maternal inheritance of the mt-DNA generally results in lack of heterocigosity. The one of gene that encoded by mitochondrial DNA is cytochrome b gene (Prusat and Grzybowski, 2004). Cytochrome b gene of several vertebrates, including mammals, were mainly investigated for evolutionary, genetic diversity and molecular phylogenetic studies (Wolf et al., 1999).

MATERIALS AND METHODS

Samples collection and DNA extraction. Both blood and ear tissue were collected from Madura Island, Pacitan Regency, Yogyakarta and another region (Ball, Kalimantan and NTE; Ball cattle). The DNA was isolated from blood samples and ear tissue. Blood samples were prepared by using DNA isolation KIT high pure PCR template preparation (ROCHE) appropriate with it’s protocol. Ear tissues were prepared by using standard SDS/proteinase K extraction (Sambrook et al., 1989).

PCR and RFLP analysis. Amplification of the mt-DNA cytochrome b (cyt b) gene was carried out in a final volume of 20 μl in 0.5 ml tubes containing 13.3 μl aquabidest, 2 μl PCR buffer, 1.5 μl MgCl₂, 0.1 μl dNTP mix, 1 μl each primer (L14735: AAA AAC CAC CGT TGT TAT TCA ACT A and H15149: GCC CCT CAG AAT GAT ATT TGT CCT CA) as universal cyt b internal primer pair, designed by Kocher et al. (1989), 0.5 units of Taq DNA polymerase and 1 μl DNA genomic. The cycling conditions were as follows: 94°C for 2 min for pre-denaturation, 35 cycles of 36 s at 95°C, 73 s at 51°C, 84 s at 72°C and followed by a final extension step of 3 min at 72°C and 4°C until the next step. PCR products were examined by electrophoresis through a 1% agarose gel in 10X TBE buffer and stained by ethidium bromide. As size reference, Novagen marker was used. PCR product was digested by restriction enzymes as described previously (Verkaar et al., 2002) and electrophoreted on a 2% agarose gel. In this work we used TaqI restriction enzymes.

RESULTS AND DISCUSSIONS

Polymorphism of mt-DNA Cytochrome b based on TaqI-RFLP Analysis

Polymerase chain reaction was carried out in a final volume 20 μl. The PCR product was resulted 464 bp. The PCR product was digested by TaqI restriction enzyme (Fig. 1).
Figure 1. The TaqI-RFLP analysis of Indonesian domestic cattle's mtDNA cytochrome b. Lane 1 is Bali cattle (similar pattern with Madura and PO cattle from Pacitan Regency), lane 2 is Local Pacitan cattle (Pc11), lane 3 is PO cattle from DIY (PO22) and lane 4 is the PCR product (464 bp) as a negative control.

Figure 1 show TaqI-RFLP pattern of Indonesian domestic cattle. The restriction patterns were grouped at three different haplotype. The first pattern generated 225 bp, 191 bp and 48 bp (lane 1), was haplotype A; second pattern generated 416 bp and 48 bp (lane 2), was haplotype C; and the third pattern generated 372 bp, 48 bp, and 44 bp (lane 3), was haplotype B. The cleaved fragment sizes were determined by individual haplotype sequences analysis with DNAMAN software. The results showed that Bali, Madura and Native Pacitan cattle included to the haplotype A (the complete data not shown), the PO cattle from DIY (Bos indicus) included to the haplotype C, while Local Pacitan cattle, especially Pc11 cattle included to the haplotype B.

Sequences Analysis of Three Different Haplotype in Indonesian Domestic Cattle

Three different individual haplotype sequences compared to the some sequences from NCBI. Sequences analysis was performed by DNAMAN software. The results showed that PO cattle (PO22) have similar TaqI-RFLP pattern with the AF492351, AF492350 and NC005971. This results indicated that PO cattle related to Zebu (Bos indicus) and Bos taurus cattle. Prado et al (2005) reported that Bos indicus and Bos taurus have similar TaqI-RFLP pattern and produced three DNA band with 372 bp, 48 bp and 44 bp in sizes (haplotype B).

One of native Pacitan cattle (Pc11) was haplotype C. It produced two DNA band with 416 and 48 bp. It's sequence and TaqI-RFLP pattern almost similar with the Bos indicus and
Bos Taurus sequences. The differentiation between them due to alteration nucleotide number 93, C→A (Fig. 2. compared lane Pc11 with lane AF492351, AF492350 and NC005971).

![Diagram showing sequence alignment with TaqI restriction site]

**Figure 2.** Polymorphism of Indonesian Domestic cattle sequences in three different haplotype. The box indicated TaqI restriction site. The sequences analysis was performed with the DNAMAN software.

The Bali, Madura and some native Pacitan (complete data not shown) cattle were included to the haplotype A. This result indicated that the cattle had the same maternal lineage (maternal inheritance). The Madura cattle suspected as a result of cross breeding between Zebu and Banteng cattle (Nijman et al., 2003; Wijono and Setiadi, 2004). The Bali cattle was domesticated banteng (Anonmous, 2005). The genetic characteristics similarity due to Bali, Madura and native Pacitan cattle contain Banteng nucleotides in it's mt-DNA cyt
b gene. It supported that Bali cattle had the same sequence and TaqI-RFLP pattern (Fig. 2) with the AY689188 (Bos javanicus/Banteng).

CONCLUSIONS

There were mt-DNA cytochrome b polymorphism in Indonesian domestic cattle based on TaqI-RFLP analysis and the study also have vary haplotype, there were haplotype A, B and C. Sequences analysis indicated that the most Indonesian domestic cattle has Banteng nucleotide in its cyt b gene sequences.

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