INTERNATIONAL CONFERENCE ON BIOLOGICAL SCIENCE

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

PROCEEDINGS

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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, sponsorships, 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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BNI UGM Branch, Yogyakarta
Thematic Oral Presentation

Topic 1: Molecular Biology, Genetics and Bioinformatics

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

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8. Study of SugarcaneSucroseTransporter cDNA by Functional Expression in Yeast
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9. Cloning and Transient Expression of Promoter from Elongation Factor 1 Alpha Gene (MeEF2) from Cassava (Manihot esculenta Carn tz.)
   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
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12. Expression of Parthenocarpic Gene, DefH9-iaaM On Transgenic Tomato Lines
    Saptowo J.Pardal, R.Purnamaningsih, E. G.Lestari and Slamet

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5. Reintroduction and Survivorship of the Threatened Palm Pinaga Javana Blume in the Gunung Halimun National Park, West Java
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6. Toward The Indonesian Redlist Book: Species Priority Setting for Conservation of Indonesian Threatened Plants
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GENETIC RELATIONSHIP BETWEEN ONGOLE HYBRID CATTLE AND BOS JAVANICUS IN INDONESIA BASE ON PARTIAL D-LOOP MTDNA

Tety Hartati, Tri Satya Mastuti Widi, Muhammad Cahyadi and Muhammad Affan Mu'in

Department of Animal Production, Faculty of Animal Science, Gadjah Mada University, Indonesia Department of Animal Production, Faculty of Animal Science, University of Papua, Indonesia

ABSTRACT

Bos indicus such as Ongole and other zebu cattle had been developed in Indonesia during Dutch Colonial period. The pure Ongole was brought to Sumba island and became the Sumba-Ongole. The Sumba-Ongole at Java had been crossed with the Javanese cattle (Bos javanicus) and formed the Ongole-grade (Peranakan Ongole). Ongole crossbred (PO) is the most number of local cattle in Indonesia. The introduction of exotic breeds (Simmental and Limosin) in Indonesia started since 1970s. We have analyzed the genetic material of the Simmental, Limosin, Ongole crossbred and the hybrids of those cattle (Simpo and Limpo) using D-loop mtDNA sequences. The sequence type of D-loop mtDNA for the hybrid of PO and exotic breeds was closer to PO than to exotic breeds. To distinguish between several type mtDNA sequence of crossbreds, the obtain PCR products were cloned to pGEM-T easy vector and cut with different restriction enzyme resulting in species specific restriction fragment length polymorphism (RFLP). We determined that Simmental and Limousin have similarity in restriction pattern of D-loop mtDNA sequence, but Limpo and Simpo have similarity in restriction pattern of D-loop mtDNA with PO. This result suggests that D-loop mtDNA sequences in Ongole hybrid are maternally inherited from Bos Javanicus, and we found three haplotypes of Ongole hybrid cattle.

Key words: D-loop, mtDNA, Bos indicus, Bos javanicus, Ongole hybrid Cattle.

INTRODUCTION

Ongole breed has been entered to Indonesia in 1906 at Java Island, Sumatera, Kalimantan, and North Sulawesi, in which crossed with local breed to improve the quality as working cattle and meat produced (Joshi and Phillips, 1953). Ongole breed are used in crossbreeding programs as they can adapt to hot and humid climate and a good type of working (Koger, 1980; Turner, 1980). As grading up the crossbreeding between Ongole with
local breed in Java island resulted cattle’s that has similarity with Ongole breed, which called as Ongole crossbred (PO=Peranakan Ongole). This was recorded in the 19th century using Ongole bulls and small sized local Java-breeds (now considered extinct) in East Java.

The local breed has a good adaptability to the harsh condition. However, there is a tendency that farmers prefer to keep crossbred cattle instead of local breed recently. The crossbreeding programs tend to overlap between breeds; two or three breeds are crossed with the local without pay attention on their blood composition. Controlling crossbreeding is needed to prevent the worst condition (Hartatik, 2005). Up to now, there are no complains reported from the farmers regarding the quality of calves produced by crossbred, because it is still in the first generation (F1). However, it must be considered that crossing with the exotic breed may have adverse effect on the second and later generation (Udo, 1994). Therefore, investigation the genetic introduction of each crossbreed produced is needed, in order to quantify genetic diversification of cattle in Indonesia.

Many crossbreeding programs using exotic bulls (Bos Taurus) or frozen semen (artificial breeding) in these regions were failed to yield desirable results (Martojo, 2005). Success occurred only where Ongole (Bos indicus) or PO crossbreeding (Bos indicus-Bos Javanicus) was utilized. This program cause the genetic mixture of Bos indicus-Bos Javanicus and Bos Taurus. There is potential to threaten the genetic identity of indigenous breed types. The aim of this research is to identify the genetic relationship between Ongole hybrid cattle (Bos indicus-Bos Javanicus- Bos Taurus) and Bos Javanicus in Indonesia.

MATERIAL AND METHODS

DNA Samples. DNA was isolated from the whole blood and ear tissue using standard SDS/proteinase K extraction, with 730 μl lysis buffer (50 mM Tris-HCl pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) and 20 μl Proteinase K (20 mg/ml) (Sambrook et al., 1989).

Polymerase Chain Reaction (PCR). A fragment of the mtDNA D-loop was amplified from 50 ng templates DNA with the primers D-loop-L (5'-AAAAATCCCAATAACTCAACACAG-3'; positions 15848-15870) and D-loop-R (5'-TACAATAGATGCTCCGGGTACAG-3'; position 126-105). PCR was performed in 20 μl reaction mixture containing 1 μl of 10 pmol for each primer, 1.5 μl of 25 mM MgCl2, 2 ml of 10x mgCl2 free buffer, 0.1 ml dNTP and 0.1 ml Taq polymerase (promega) with the following program: predenaturation for 2 min at 92°C, 30 cycles of 15 s at 92°C, 45 s at 52°C, 45 s at 72°C and followed by a final extension step of 7 min at 72°C and 4°C until the next step. PCR product were seperated by 1% agarose gel electrophoresis in 1x TBE buffer and the
excised fragments were purified with the QIAquick isolation system (Qiagen, USA) and inserted in the pGEM-Teasy vector (Promega, USA). Clones were purified with the QIagen Midiprep kit and 300 ng of pGEM Teasy positive clones were sequenced using T7 and SP6 primers with the following program: 96°C 1 min, 25 cycles of 96°C 30 s, 50°C 15 s and 60°C 4 min, BigDye Terminator v3.1 Cycle sequencing Kit and an ABI Prism™ 310 sequencer Systems (Applied Biosystems).

Sequence analysis. Bos Javanicus sequences were retrieved from the NCBI with Blast search. The appropriate database from Genbank was compare to the partial D-loop mtDNA in this study. The alignment of the sequence was analyzed by the program Bioedit (Hall, 2002) and homology tree was constructed by using DNAMAN program.

RESULTS AND DISCUSSION

The DNA samples were consist of 5 samples of PO, 3 samples of Limpo, 5 samples of Simpo, 4 samples of Limousin semen and 4 samples of Simmental semen. Figure 1 shows the process to obtain the positive mtDNA D-loop clone in pGEM-T easy vector. The PCR product of mtDNA D-loop (Figure 1A) shows 600 bp. After purification with QIAquick isolation system (Qiagen, USA), the PCR products were cloned into pGEM-T easy vector (Figure 1B). The analysis of mtDNA D-loop with Ssp1 restriction enzyme was described at the Figure 1C. Analysis sequence of mtDNA D-loop using Ssp1 restriction enzyme shows that the pattern of restriction site for Simpo, Limpo and PO (lane 2, 3, and 4) was similar, another pattern also found at restriction site for Limosin (lane 1) and Simmental (lane 5) compare with the control of pGEM-Teasy vector (lane 6). Thus, identification of Ongole hybrid cattle (Bos Indicus-Bos Javanicus) compare with Bos Taurus can be recognized by Ssp1 restriction enzyme.

![Figure 1](image1.jpg)

Figure 1. Polymerase Chain Reaction (PCR) product of partial D-loop mtDNA (A), cloning the PCR product into pGEM-T easy vector (B) and digestion with Ssp1 restriction enzyme(C).
Base on partial mtDNA D-loop sequence, there are three haplotypes of Ongole hybrid cattle (see Table). The mtDNA D-loop sequences of Ongole hybrid cattle were compared to the *Bos indicus*, *Bos taurus* and *Bos javanicus* mtDNA D-loop sequences from NCBI. There are three groups based on homology of mtDNA D-loop sequences. The first sub tree was *Bos taurus* group. The second sub tree was *Bos indicus* group, where is almost all of Ongole hybrid cattle included in, and the third group was *Bos javanicus* group.

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<td>I</td>
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Hyphen indicates the identical nucleotide with reference sequence.

* Number written vertically show nucleotide base position of mtDNA D-loop.
* Reference sequence Zebu (AF1262484).
* Number in parenthesis indicate number of animal observed.

OH = Ongole Hybrid, OHS = OHxSimmental, OHL = OHxLimousine

Table: D-loop haplotype based on partial mtDNA D-loop sequence

The results showed that all Ongole hybrid cattle samples does not include in *Bos taurus* group. It indicated that mtDNA D-loop was inherited by maternal line. Therefore, almost all of Ongole hybrid cattle have genetically homology to the *Bos indicus*. Since Ongole hybrid cattle were derived from *Bos javanicus*, *Bos indicus* and *Bos Taurus*, some of Ongole hybrid cattle were included in *Bos javanicus* group. These results suggest that a part of Ongole hybrid cattle has genetically identity with *Bos javanicus*. 
Figure 2. Homology Tree of Ongole-hybrid cattle.
Mitochondrial DNA is a marker of maternal lineage, which in cattle corresponds to the history of the herd (Nijman et al. 2003). So cows of the hybrid of Simpo and Limpol were used from Ongole hybrid. Exotic breeds were introduced to Ongole hybrid mostly by artificial insemination (Hartatik et al. 2006). The results were reliable, because almost of the mtDNA D-loop sequences of the hybrids revealed similarity mtDNA D-loop sequences of the Ongole hybrid. Our data indicate that the Ongole hybrid cattle closer to Bos indicus than to Bos javanicus. This explorative observation may become essential molecular basic data for the future breeding plan in cattle.

REFERENCES