

INDONESIAN BIODIVERSITY AND BIOTECHNOLOGY: LIPI Update

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ABSTRAK

Indonesia adalah negara dengan keanekaragaman hayati terbesar di dunia (mega diversity country). Negeri ini sudah sepantasnya memiliki nilai tinggi bagi keberlanjutan kehidupan umat manusia di atas planet bumi yang sangat rapuh ini. Keanekaragaman hayati adalah satu-satunya sumber materi genetik yang diperlukan untuk menumbuhkan bioteknologi. Keanekaragaman hayati dan sumber daya genetik yang terkandung di dalamnya diyakini akan sangat penting dalam upaya manusia menyesuaikan diri dan mengantisipasi perubahan iklim global dan memastikan pencapaian target MDG. Sebagai negara dengan keanekaragaman hayati terbesar, Indonesia berpeluang sangat besar untuk memberikan kontribusi bagi kepentingan umat manusia tanpa kecuali. Melalui kemajuan ilmu biologi, khususnya di bidang biologi molekuler dan genetika molekuler, keanekaragaman hayati dan genetik yang ada di dalamnya dapat terus dipelajari, dilestarikan, dan dimanfaatkan untuk berbagai kepentingan, baik pertanian (produksi benih yang adaptif terhadap perubahan iklim dan perubahan pola konsumsi), industri (bioekonomi/ekonomi hijau), kesehatan (produksi obat baru untuk mengatasi berbagai penyakit baru/new emerging diseases), dan lingkungan (meningkatkan kualitas lingkungan). Lembaga Ilmu Pengetahuan Indonesia (LIPI) telah mendirikan Pusat Penelitian Bioteknologi, 10 tahun sebelum dibentuknya Komite Bioteknologi Nasional. Melalui sejarah panjang, LIPI secara berkesinambungan terus melakukan kegiatan riset di bidang keanekaragaman hayati. Dengan komitmen penuh, LIPI berupaya merawat koleksi spesimen tumbuhan, hewan, dan mikroba di Herbarium Bogoriense, Museum Zoologicum Bogoriense, Koleksi Kultur Mikroba, dan Kebun Raya, termasuk 18 Kebun Raya baru yang didirikan di berbagai kota dan provinsi di seluruh Indonesia. Penelitian terhadap nilai manfaat keanekaragaman hayati juga dilakukan. Penelitian molekuler dan genetik merupakan salah satu di antaranya. Beberapa telah menunjukkan hasil yang positif. Kerja sama ilmiah internasional menjadi salah satu hal yang harus dilakukan dalam mempercepat pemahaman tentang arti pentingnya keanekaragaman hayati dan genetik yang ada di dalamnya serta mencari mekanisme pemanfaatannya yang tepat sesuai dengan kesepakatan internasional (CBD, Protokol Cartagena, dan Protokol Nagoya) sebelum sumber daya alam hayati ini mengalami kepunahan.

Kata kunci: *Keanekaragaman hayati, Sumber daya genetik, Bioteknologi, Bioteknologi tumbuhan, Therapeutics protein, Gen tahan flu burung*

INTRODUCTION

Indonesia is the largest archipelago in the world, comprised of more than 17,000 islands. It lies on the tropical belt with diverse ecosystems and terrains, ranging from the deep ocean, low lands and mountain jungles, to snowy peaks. Indonesia also houses of nearly 17% of living creatures in the world, 10% of flowering plants, 12% of mammalian, 25% of reptiles, and countless of un-explored microbial and genetic resources. As

a mega-diversity country, Indonesia is the richest genetic resources in the world. Those biodiversity treasures are very important elements as the fundamental resources for future advancement in biotechnology research and application for the benefit of humanity.

With the advancement of biological sciences, particularly in the field of molecular biology and molecular genetics, these diverse genes may be studied, isolated, amplified, preserved, and

utilized for various purposes. The utilization of genes through advanced techniques of biotechnology offers great potential applications for agriculture (production of food), industry (added value of agricultural products), health (traditional medicine and drug development), environment (improve the quality of environment), and serves a vehicle for the adaptation to global climate change and ensuring the achievement of MDGs.

The Indonesian Institute of Sciences (LIPI) has foreseen the importance of biotechnology for the future of Indonesia. Ten years prior to biotechnology research was included into national priority agenda in the late 1980's. LIPI has established Research and Development Center for Biotechnology.¹

In the late 1990's Indonesian Government set up a National Committee for Biotechnology to prepare and formulate a national biotechnology policy and to develop strategic program to assist national development. The Committee also provides guidance and encourages the development of bio-industry and its supporting R & D and human resources. It gives directions for the establishment of national, regional, as well as an international network of cooperation on biotechnology, and to monitor the implementation of the national policy on biotechnology.²

To support a vast research in biotechnology, LIPI has put priority for its human resource development, by providing a wide range of opportunities for young scientists to pursue advanced education overseas, through various programs in Japan, Australia, Europe, and North America. To implement the policy, a program has been formulated with the following priorities: production of fine chemicals and pharmaceuticals, such as antibiotics, amino acids, vitamins; mass production through micro-propagation of industrial, horticultural, and forestry plant species; improvement of food crops quality, in particular rice and soybean; improvement of beef and dairy cattle quality through embryo transfer, and production of various diagnostics and vaccines for human and animal diseases. For this purposes, Indonesian Government has assigned Research Institute for Food Crops and Research Center for Biotechnology–LIPI as Centers of Excellence on Agricultural Biotechnology. In the

field of health, Faculty of Medicine–University of Indonesia was set up as Center of Excellence on Health Biotechnology, while Agency for Technology Assessment and Application – BPPT was assigned as Center of Excellence in Industrial Biotechnology.

Each of these centers has the task to set up a network of institutions working on similar fields. The Government of Indonesia also established Inter University Center on Biotechnology in three universities, namely Bogor Agriculture University in Bogor focused on Agriculture Biotechnology, Bandung Institute of Technology in Bandung focused on Industrial Biotechnology, and Gajah Mada University in Yogyakarta focused on Health Biotechnology.

BIOTECHNOLOGY ACTIVITIES

For the last three decades, the Government of Indonesia has consistently provided grants for prominent researchers in the field of biotechnology through various competitive research grants. Indonesian Government also improve the management of research through one gate policy, revitalized the National Research Council and established a panel of expert to help the government in selecting proposals for funding through various funding mechanisms. Since then, biotechnology research activities in Indonesia is flourishing. Schmid, R. D. et al.³ noted that a number of Research Institutions of various affiliations have been identified to carry out research activities in biotechnology, including:

1. Universities: Faculty of Pharmacy, University of Airlangga–Surabaya with special emphasis on plant cell cultures, biotransformation with plant cells, rat hepatocyte cultures; Food and Nutrition Development Centre and Research Centre UGM–Yogyakarta on biopreservation, lactic acid bacteria, cell fusion among *Aspergillus* strains, monoclonal antibodies for aflatoxin; Inter University Center for Biotechnology UGM Yogyakarta on genetic analysis of Waardenburg syndrome, Thalassemia, Dengue viral antigens, diagnostic tools based on PCR, erythromycin and BT toxin production; Inter University Center on Biotechnology of ITB–Bandung on microbial fermentation, enzyme technology,

- genetic engineering, biological waste water treatment; School of Medicine Airlangga University – Surabaya on reproductive health, infectious diseases, cancer and degenerative diseases, forensic serology; Department of Microbiology UI–Jakarta on dengue virus diagnostics, Salmonella diagnosis, hepatitis C research; Faculty of Agriculture UGM–Yogyakarta on Baculo virus detection, CVPD-free citrus seedlings, PCR technology, SMZ coat protein genetics for virus-free soybean stocks, food biotechnology; Inter University Center for Biotechnology IPB–Bogor on the improvement of plant productivity by tissue culture, embryo transfer, microbial biotechnology, waste treatment, culture collection;
2. Research Institutes: Indonesian Sugar Research Institute (P3GI)–Pasuruan, East Java on dekstranase, xanthan gum, sugarcane breeding, wastewater treatment, genetic engineering techniques; Central Research Institute for Food Crops, Laboratory of Plant Biotechnology Bogor of the Department of Agriculture–Bogor on molecular genetics of rice diseases, cell and tissue culture, nitrogen fixation, bio-fertilizers, bioconversion; Marihat Research Center Pematang Siantar North Sumatera on tissue culture on cocoa, rattan, vanilla, oil palm etc.; Research Institute for Animal Production (Balitnak) of the Department of Agriculture, Ciawi–Bogor on feed improvement using fermentation, mannase, embryo transfer, phytase, cassava-protein; Research Institute for Veterinary Science (Balivet) of the Department of Agriculture–Bogor on cloning of veterinary toxins, veterinary immunology, monoclonal antibodies; Institute for R & D of Agro-based Industry of the Department of Industry–Bogor on industrial biotechnology, fermentation of soybean curd whey, food quality control; Center for the Assessment and Application of Technology (BPPT)–Jakarta on antibiotics production, plant, dish and livestock production, vitamin, enzyme and amino acid production; Research Center for Applied Chemistry–Indonesian Institute of Sciences (LIPI)–Bandung on bioconversion of solasodine, waste water treatment, fermentation, tempe; Research Center for Biotechnology–Indonesian Institute of Sciences (LIPI)–Cibinong, Bogor on fermentation and enzyme technology for production of enzymes and biocatalists, plant biotechnology (genetic analysis and transformation), embryo (production/ preservation/manipulation/transfer technology), aquaculture, natural products; Eijkman Institute for Molecular Biology–Jakarta on mitochondrial DNA mutation in human diseases, ageing process, energy-transducing systems, thalassemia, diagnostic kit for Dengue hemorrhagic fever;
 3. Private Company: Perum Bio Farma (State Enterprise)–Bandung on vaccines, sera, diagnostics; PT Kalbe Farma (Indonesian Pharmaceuticals Company)–Jakarta on diagnostics; PT Meiji (Japanese Pharmaceutical Industries operated in Indonesia)–Jakarta on antibiotics; PT Rhone-Poulenc (French Pharmaceuticals Company operated in Indonesia)–Jakarta on vaccines; PT Sandoz Biochemie (Swiss Pharmaceutical Company operated in Indonesia)–Jakarta on antibiotics; Pusat Veterinaria Farma Surabaya (State enterprise) on vaccines, and antigens; PT Sasa Inti Probolinggo (Indonesian) on glutamic acid; PT Ajinomoto Mojokerto (Japanese) on glutamic acid; PT Miwon Indonesia Gresik (Korean) on glutamic acid; PT Indo Acidatama Surakarta (Indonesia) on ethanol; Perusahaan Daerah Aneka Kimia Surabaya (State Enterprise) on ethanol; and Rhizogin Indonesia Jakarta/Bogor (Indonesian) on Rhizobium starter cultures.

NEW CHALLENGES FOR LIPI

During the last two decades, Indonesian Institute of Sciences (LIPI) has developed infrastructure to support research in the field of life sciences, including and in particular biotechnology. Infrastructure development for biotechnology within LIPI is centralized in Cibinong Site, some 25 kilometers south of Jakarta. This site covers an area of 190 hectares and was dedicated by the late President Soekarno in 1959 for science and technology development center to support Indonesian development plan (Pembangunan Semesta Berencana). But not until mid 1990s Cibinong Science Center of LIPI was established. Since then, LIPI has been expanding biotechnology research activities from fermentation and enzyme production technology, plant biotechnology (genetic analysis and transformation), embryo

(production/ preservation/manipulation/transfer technology), aquaculture, and natural products toward molecular genetic studies.

After 20 years of experience, it is now possible for LIPI to strengthen its biotechnology capacity. Various strong research groups within the institutes are flourishing, which lead to the rapid development of collaborations, including collaborations with international scientific communities and attract funding from various funding agencies.

MICROBIAL BIODIVERSITY EXPLORATION

A collaborative research on taxonomy of fungi and actinomycetes between Indonesia and Japan has been carried out between 2003 and 2006, and it is extended to 2009. This joint research was conducted to provide a model for the implementation of CBD, i.e. to provide solution in sustainable use of biodiversity while exploring its benefit for human welfare. Research cooperation was conducted on mutual trust and mutual respect among Indonesian and Japanese scientists under the umbrella of MoU and Technical Agreement. The aim of cooperation was to establish appropriate mechanisms for conservation and sustainable use of microbial resources for scientific and industrial purposes. Diverse types of samples, with mainly soil and plant litter, from 13 locations in Indonesia were collected and the isolation of fungi and actinomycetes were carried out. Seventeen methods of isolation for fungi, two methods for yeast and six methods for actinomycetes were employed. The total number of isolated and identified microorganisms generated from our research cooperation include 2,507 (205 genera) of fungi, 515 (27 genera) of yeast and 3,193 (68 genera) of actinomycetes. Other important output of the project includes data and information on microbial diversity, up grading of human resources and open possibilities in developing a new project. The results have been reported in various symposia and published in international journal including *Mycoscience* and *IJSEM* describing new taxa. Benefit sharing arising from this project also include other non monetary as well as monetary. Through further collaboration with Hokaido University, our scientist found one of Actinomycetes isolate

which could be use as a candidate for the production of enzymes responsible to convert inulin to DFA-III which have a great potential value to accelerate calcium transport in the blood stream. It is hence has a tremendous potential for preventing osteoporosis. Patent document for this process is in progress.^{4,5,6,7}

It is also interesting to note the results of cooperation between LIPI and University of California Davis on exploration of microbial diversity of Mekongga, SE Sulawesi, for health and energy solution. This highly interdisciplinary project was funded through the NIH International Cooperative Biodiversity Groups program. The aims are to perform a biodiversity survey in the Mekongga Mountain Range in Indonesia, and to use the data to improve conservation policies, screen biological materials for new therapeutics, and screen for bio-energy applications. We have screened microbes for both high lipid content and cellulase activities. We have isolated over 1,500 microbes to date from our research sites, including bacteria, actinomycetes, yeasts, and filamentous fungi. A portion of the microbes were isolated from wood-feeding beetle larvae, because these larvae feed exclusively on wood. We targeted recently killed, upright trees, which have not been heavily infested with wood decay fungi, with the hope that these beetles carry in their guts microbes with powerful cellulases. We also have isolated microbes from the infestation galleries and tunnels, as well as decaying wood. There are likely some novel species of bacteria, filamentous fungi and yeasts. Those will be characterized and put on publication. To date, of the ribosomal sequences of 129 yeast strains, 34 are likely new species based on divergence in the D1/D2 region of the large (26S) ribosomal subunit. These 34 strains represent 20 different species. We selected for characterization 20 strains belonging to six different species clustering in the *Candida intermedia* clade.

We are now beginning to characterize the cellulolytic activity of these microbes. We have assayed over 160 microbes for ability to hydrolyze carboxymethylcellulose. While a large proportion of filamentous fungi were positive, small proportions of yeasts and bacteria were

able to hydrolyze this substrate. We have begun assays on AZCL-conjugated substrates.

AVIAN FLU RESISTANT GENES IN INDONESIAN CHICKEN VARIETIES

Indonesian indigenous chickens throughout the archipelago apparently have a certain degree of diversity with different morphologic characteristics. There are 31 breeds of indigenous chickens which have been identified. Samples have been taken from a total of 483 individuals belonging to 15 different populations. The Mitochondrial DNA displacement (D)-loop sequences were used to study the genetic diversity and relationship of Indonesian indigenous chickens. Phylogenetic analysis indicates that Indonesian indigenous chickens can be grouped into five distinct clades (clade I, II, IIIc, IIIId and IV) of the previously identified seven clades (clade I, II, IIIa, IIIb, IIIc, IIIId and IV) in Asian indigenous chickens. The Indonesian chickens are different from indigenous chickens from other Asian countries because it dominantly belongs to clade II. Based on the composition of chicken clade in Asia, it shows that three large countries have very special composition of clades, i.e. Indus valley area is dominated by the population of clade IV; Yellow River, Henan is dominated by the population of clade IIIId, and Indonesian area is dominated by clade II. It is concluded that Indonesia is one of three countries which are centers of chicken domestication.⁸ Recent finding shows that this relationship is importance for assessing the possible impact of outbreaks of viral diseases, such as avian influenza, on the diversity of the indigenous Indonesian chickens.

PLANT BIOTECHNOLOGY

A group of researchers at Research Center for Biotechnology, LIPI, currently has the capacity to conduct research on gene hunting. They are currently focusing on the identification and study of gene responsible for biotic and abiotic tolerant mechanisms, and its application to improve plant tolerance against those stresses. We are applying transposon tagging with gene-trap and activation-tag to develop mutant population as screening materials. We have generated thousands of stable mutant lines and are currently being

screened for drought, salt, and shade tolerances. Recently, we have identified mutants lines with improved tolerance under drought and salt stress. The molecular mechanisms underlying those mutation are still being investigated. We are also studying some potential genes and transcription factors (TF's) responsible for drought resistance in our attempt to develop drought resistant lines. Some of the TF's have been studied on model plant *Arabidopsis thaliana*,⁹ while studies on rice using putative consecutive and inducible promoters are underway. Our lab is the first in Indonesia to perform limited field trials of locally developed genetically modified rice. In 2007 we have completed a series of biosafety tests of our Bt rice in collaboration with the Department of Agriculture, which cover environmental safety aspects, such as impact to non-target insects.¹⁰⁻³⁴

In addition to crop improvement against biotic and abiotic stresses, we are also in an attempt to develop hepatitis B surface antigen edible vaccine expression system in banana.

PROTEIN THERAPEUTICS

Biotechnology provides tools in solving diverse human dilemma, in particular through the production of DNA recombinant base drugs. The process is dominated by developed countries. Indonesia is 100% dependent on import. Our aim is to develop the capacity to produce protein therapeutics. Research on hEPO is highly demanded in the country, but the cost is very high and not always affordable. Human erythropoietin (hEPO) is an important glycoprotein in human that is coded by a single gene named EPO (erythropoietin). EPO is a glycoprotein hormone that promotes erythropoiesis, which is the formation process of mature red blood cells (erythrocytes) in human bodies. It is widely used for treatment of anemia in patients with chronic renal failure. Recombinant hEPO (rhEPO) is produced in mammalian cells, such as Chinese Hamster Ovary (CHO) cells for the reason of its complex structure as a glyco-protein. In an effort to use and optimize heterologous EPO gene expression in an alternative eukaryotic host cells such as yeast, an EPO-synthetic gene (EPOsyn) was constructed. The synthetic gene had been designed to contain optimal usage of *Pichia pastoris* codon. It had been constructed

by a recursive-PCR method in two-step PCR reactions. The gene was assembled from eight single strands synthetic oligonucleotides having an average length of 90 nt with 20 to 30 overlap region between two adjacent oligos. The synthetic gene has less GC content (45.31%) compared its native (human) gene (59.08%). The synthetic gene has been cloned in pCR2.1 cloning plasmid and sequenced. From eight independent clones, it was revealed that the error rate was 1.59%, in which 1.42% was due to deletions and 0.17% due to substitutions.³⁵

Research group at Research Center for Biotechnology LIPI has successfully isolated and expressed gene responsible for the production hEPO on Barley Mozaic Virus and *Pichia pastoris*. This achievement identified as the first necessary step serves as foundation for Indonesia to develop protein therapeutics based on DNA recombinant technology.

CONCLUSION

Indonesia should improve her management capacity to conserve biological diversity and it expands her capacity in studying biological diversity at ecosystem to molecular genetic level to gain most benefit for the prosperity of humankind while protecting the balance of nature and human interest. The achievement should be communicated to the society and policy decision makers to increase awareness of the value of biological and genetic resources for next generation.

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