Second Science Symposium

August 11, 2004

Alawattagoda Pemadasa Auditorium
Faculty of Science, University of Ruhuna

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Proceedings of the Second Science Symposium

August 2004

Faculty of Science
University of Ruhuna
Matara, Sri Lanka

ISSN 1391 - 8796

Preface

The Faculty of Science, University of Ruhuna is proud to present the Proceedings of its Second Science Symposium. The members of the faculty are engaged in many research activities in their respective fields of interest and findings are published regularly in national and international journals. However, the Science Symposium offers a great opportunity for academics in the faculty to present their research findings to an audience largely consists of their own colleagues. This encourages other staff members as well as students in the faculty to engage in more and more research activities, especially, leading to inter-disciplinary collaborations.

This proceeding consists of nine papers from research fields in Botany, Chemistry, Mathematics, Physics and Zoology. Cobra-repellent activity by "Andu" and "Agil" plants, a possible rainfall forecasting model for Sri Lanka using a neural network, a population dynamics study of some reservoir fishery species in three reservoirs of southern Sri Lanka, an investigation of the potential for using mitochondrial protein coding gene regions for studying geographic patterns of genetic divergence among marron populations in western Australia, ecological role of aquatic macrophytes on micro-ecosystem diversity of pondweed beds in Embillakala lagoon of Bundala national park, changes of mangrove cover of Rekawa lagoon, rare events in T-Cell activation, inhibition of growth of antibiotic-resistant *Staphylococcus* sp. and *Proteus* sp. by mangrove plant extracts and suppression of Collar-rot caused by *Sclerotium rolfsii* Sacc. in chilli by antagonistic bacterial treatments are the studies presented in the research articles published in the current proceeding.

The editorial board thanks all authours who contributed by submitting papers for the symposium. Our special and sincere thanks go to selected eminent scholars from other universities and research institutes for refereeing the articles. The faculty wishes to conduct the symposium as an annual event to facilitate publication of research findings of the staff thus promoting research activities of the academics in the faculty further.

Editorial Board
Science Symposium - 2004
Faculty of Science
University of Ruhuna
Matara, Sri Lanka.

Keynote Address

From Research to Industry: Molecular Diagnostics in Sri Lanka

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Universities can be considered as places of independent thought and research. Major breakthroughs and discoveries in science have been made by researchers attached to universities. As a young postdoctoral scientist, with so much enthusiasm, I joined the academic staff of the University of Colombo to teach and to conduct research in the field of molecular biology, a subject I have been fascinated by and associated with since my post graduate training. Soon after joining the university, I considered myself extremely lucky when the faculty of science was awarded a JICA grant to upgrade facilities for teaching and research and I was able to acquire a fully equipped molecular biology laboratory – a dream come true. Research grants secured from the Third World Academy of Sciences, Italy, International Foundation of Science, Sweden, International Atomic Energy Authority, Austria and the National Science Foundation, Sri Lanka, and the immense contributions made by the research students of mine made it an active research laboratory. International and local awards that we have won for our research work proved that more than the presence of big buildings and sophisticated equipment, the key to success is the people and teamwork. Today I would like to dedicate this lecture to all my former undergraduate and postgraduate research students particularly to my three Ph.D. students (Himesha Vandebona, Neil Fernandopulle, Yasantha Mapatuna) who went through a path of obstacles during the establishment of the laboratory and yet they did not give up till they reached their final goal – the Ph.D. Their research findings not only generated publications and awards, but also led to applications - much needed molecular diagnostic services in Sri Lanka: Having established a research laboratory, we were able to go one step further and offer limited services to the public such as DNA typing services for criminal and civil case work (fee levying) and a diagnostic test for dengue virus (IAEA funded) using in-house developed test kits. However, my vision was to have a self-funded, fully functional and an efficient university center offering molecular diagnostic services to the public at a large scale. Unfortunately I could not realize this goal as my vision of the future at that time was not visible to many. This set-back did not discourage me and with much help and support from my husband Dhammika and my former Ph.D. Student Dr. Neil Fernandopulle, what I could not achieve in the university we achieved outside the university. We now have a molecular diagnostic facility (Genetech Molecular Diagnostics) in Sri Lanka offering disease

diagnostic services and DNA typing services for paternity testing and criminal casework, at a fraction of cost that would have incurred if these services were sought abroad. We have provided employment to 14 personnel including 4 post-graduates with Ph.D/M.Sc. qualification and 4 B.Sc. graduates. Through the same facility (Genetech School of Gene Technology) we have also trained more than 200 in the field of molecular biology and more than 1000 police officers in the use of DNA evidence during the last two years. Up to now we have performed DNA paternity testing for more than 250 cases of child maintenance cases and developed in-house molecular diagnostic tests for more than 25 infectious diseases including tuberculosis, dengue, hepatitis B and C, Cytomegalovirus, Japanese encephalitis virus, Herpes simplex virus, AIDS virus and malaria, microfilaria, Leishmania parasites and genetic disorders such as thalassemia, Down syndrome and DMD as well as for leukemia screening. At the inception of Genetech, the public was not aware of molecular diagnostic tests, however, by the end of my talk you would realize how we have created a Sri Lankan market for the applications of modern technology. Today I have a great sense of satisfaction and feel that I have paid for my free education by making the benefits of gene technology accessible to the wider public of Sri Lanka thereby fulfilling a national need.

The atmosphere in the universities is more conducive for research now than then. Over the past two years, university authorities have realized by offering fee levying services to the public sector, funds can be raised to maintain their laboratories and the Science and Technology Development project funded by the ADB is committed to help the universities to achieve this goal. Today I would like to share my experiences with you in developing a molecular diagnostic facility and show you the wide repertoire of diagnostic methods currently available, with the intention of inspiring those young researchers and the decision-makers of the universities. By demonstrating to you how findings of research could be turned into industry, I hope it will help and motivate you to achieve something that I could not achieve within the university and hope that your path to success would be of a lesser struggle than mine.

The principle of molecular diagnostic methods

Information governing the characteristics of an organism is stored in its genetic material, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA; in the case of a few viruses). Molecular diagnostics refer to laboratory diagnosis of diseases and identification of biological samples by the analysis of DNA/RNA present in living organisms. In molecular terms, a disease can be due to the entry of a pathogen i.e. any foreign DNA/RNA of an infectious agent into the body (infectious diseases); an inherited genetic disorder where the normal gene is altered/defective (genetic diseases); a sudden change

in a gene responsible for the synthesis of a protein and ageing and/or destruction of cells responsible for the synthesis of a particular protein. Infectious diseases are caused by a wide variety of organisms, ranging from multi-cellular parasites and fungi to unicellular bacteria and non-cellular viruses. Although they show extreme diversity in morphology and behaviour, what they all have in common is the possession of DNA or RNA as their hereditary material. This DNA (or RNA) also forms the basis of their individuality and their diversity, thus enabling their identification by nucleic-acid based diagnostics. Unlike other diagnostic methods, which rely on microscopy, culture, biochemical characteristics and indirect antibody detection, molecular diagnostic methods seek evidence of a disease at the very basic causative level by tracing the differences in the morphological characteristics of organisms to the differences in their nucleic acids. Similarly, genetic aberrations caused by mutations or changes in the DNA base sequence of a gene, which may in turn alter the structure and functioning of a protein or may even completely disrupt the production of a protein, can be identified by the analysis of the DNA of the tested individual. Molecular diagnostic tools are also used for identification of human biological samples. Since a person's DNA is unique (except in the case of identical twins), DNA typing/profiling enables the identification of individuals in a population, and also since a child inherits one half of his DNA from the mother and the other from his father, DNA typing can be used to determine family relationships.

Due to advances in genetechnology it is now possible, not only to clone a DNA fragment from any organism, but also to determine the complete nucleotide base sequence of the DNA fragment. Further, development of a novel technique known as Polymerase Chain Reaction (PCR) -based amplification of nucleic acids, a procedure that simply mimicks *in-vivo* DNA replication, has allowed the detection of minute amounts of nucleic acid sequences from tissue or body fluids. PCR can make millions of copies of a DNA or RNA "target" (the segment of nucleic acid to be amplified) sequence in a matter of hours, using 'oligonucleotide primers' specific to the target region to initiate DNA synthesis, substrates of DNA synthesis, the deoxynucleotide triphosphates, and an enzyme capable of DNA polymerization (eg: Taq DNA polymerase). Due to its higher sensitivity, higher specificity and accuracy, coupled with its shorter throughput time compared to the other standard laboratory diagnostic methods, PCR-based technology has dramatically changed the approach to the laboratory diagnosis of many diseases.

Diagnosis of infectious diseases

Each organism has regions within its DNA unique to the specific organism. Using DNA cloning techniques many gene/DNA targets which are either universal [e.g.: ribosomal

RNA (rRNA) and house keeping genes] or species specific (e.g.: unique 16S and 23S rRNA sequences) have been identified from many pathogenic organisms and the DNA sequence data of such cloned DNA fragments are currently available in DNA sequence databases. Using such DNA sequence information specific oligonucleotide primers can be designed for a PCR-based diagnostic assay to rapidly detect the presence of pathogenic organisms present in a clinical specimen. Among the detection methods of bacterial pathogens, the laboratory diagnosis of mycobacterial infection is an outstanding example for highlighting some of the shortcomings of standard laboratory procedures. Despite a number of effective drugs eradication of tuberculosis (TB) has not been achieved and, on the contrary, a resurgence of TB has recently been observed. According to the WHO, TB still kills 3 million individuals per year making it the leading infectious cause of death. Culture and biochemical identification methods, when properly applied detect *M. tuberculosis* (MTB) in clinical samples, with reasonable sensitivity. However, primarily due to the slow growth of the bacteria, these methods usually require 4-8 weeks or at least 10-14 days with automated radiometric system. This results in numerous delayed or missed diagnoses, adversely affecting patient care and TB control and allows for spread of the disease. Microscopy still remains the only truly rapid and widely applied standard diagnostic method for TB. However, the method is limited by its low sensitivity and the difficulty in differentiating MTB from other mycobacteria in acid-fast stained smears. PCR-based test on the other hand has had a great impact on the rapid diagnosis of TB. Detection of the organism can be done simply by **PCR-AGE** (agarose gel electrophoresis) which involves PCR amplification and visualization of the specific PCR product by AGE. During AGE the DNA fragments are separated in an electric field in a gel, according to their lengths (size). The target PCR product is identified by its specific size determined using a known DNA molecular weight marker. The specificity of PCR-based assays for MTB is excellent (>98%). Although these assays cannot replace cultures, the ability to determine rapidly (within 24 hours) the presence of MTB directly from respiratory tract specimens and cerebrospinal fluids has enabled rapid institution of effective therapy and implementation of control programmes without any delay. Some examples of other bacterial pathogens for which PCR assays have been developed and widely used in the world include *Chlamydia trachomatis*, *Chlamydia pneumonia*, *Neisseria gonorrhoeae*, *Streptococcus pneumonia*, *Bordetella pertusis* and *Mycoplasma* spp. Similarly PCR based assays are used to detect blood parasites such as malaria parasites including *Plasmodium falciparum*, *Leishmania*, spp. and filarial worms, particularly in specimens of low parasitaemia and the parasites may not be detected by microscopy . Similarly the method is also commonly used for the detection of viruses in clinical specimens. The DNA viruses such as hepatitis B,

cytomegalovirus (CMV) and herpes simplex (HSV 1&2) are detected easily by PCR-AGE. The method is used frequently for the early detection and monitoring of CMV in transplant recipients. In order to detect RNA viruses such as dengue or hepatitis C, a modified PCR procedure referred to as **RT-PCR** (reverse transcription –PCR) is employed. The RNA is first extracted from the sample and a DNA copy of the RNA (reverse transcription) is made by an enzyme called 'Reverse Transcriptase'. The DNA copy is then amplified by PCR for detection by PCR-AGE. The traditional dengue tests include the analysis of antibodies (Immunoglobulin IgM/IgG) in the blood sample or detection of virus by culture. The IgM antibody test is positive only after about 5 days after the onset of fever. For the clinical management of dengue it is important to detect the virus as early as possible. Using RT-PCR method, the virus is identified within the first day of fever. RT-PCR is also used to detect corona virus associated with the newly identified disease 'SARS'. A novel PCR-based assay known as **Multiplex PCR** enables the detection of several organisms present in a single clinical specimen. In other words the assay can also be used to screen a specimen for five different organisms simultaneously. For example, five different enteropathogens *Campylobacter, Shigella, Salmonella, Yesinia* and *E. coli,* which could be present in a stool sample are identified using a single assay. The DNA is extracted and amplified using five pairs of primers. Each primer pair is specific for a given pathogen and generates a PCR product which is also specific for the given organism. The size of the PCR product is different from one another enabling the detection of each organism without any ambiguity. Multiple infections give multiple bands in this assay. These have been used to detect common bacterial and viral causes of respiratory tract infections and meningitis. For example, a cerebrospinal fluid (CSF) sample can be screened for the presence of many possible pathogens in one assay.

The sensitivity and specificity of a PCR-based assay can be further enhanced by using a DNA "probe" to detect the amplified product in a nucleic acid dot-blot hybridization assay (**PCR-HYB**). A DNA probe (a labeled DNA fragment) or an oligoprobe (labeled oligonucleotide sequence) which will bind specifically by complementary base-pairing with an internal DNA sequence in the target PCR product is used. The DNA is blotted on to a solid support such as a nylon membrane and hybridized with the labeled probe. The unbound probe is washed away and the bound probe is detected by the signal that is emitted by the probe. Depending on the label, the detection procedure will vary (e.g.: radioisotopic label such as ^{32}P is detected by autoradioagraphy, a non-radioactive label such as biotin by colorimetry, fluorescien and digoxygenin by chemiluminescence, etc.). Another advantage of PCR-HYB method is that a large number of samples

(approximately 96 samples) can be analyzed simultaneously in a single detection assay. Another more recently developed technique is **PCR-ELISA** (Enzyme linked immunosorbant assay) which combines PCR-HYB with ELISA in a single analytical technique. Micro-titre plate format of ELISA also makes it a very rapid, convenient and sensitive diagnostic technique. The major advantage of the method is that it allows for the quantitative measurement of infectious organisms and particularly useful for the monitoring of drug therapy in patients with HIV (AIDS), hepatitis C and cytomegalovirus infections.

Detection of genetic diseases

DNA-based tests have become the diagnostic methods of choice for inherited diseases and over 200 genetic disorders can be diagnosed this way. Characterization of the human genome too has enabled the identification of many novel genes responsible disease. For example, the genetic disorder Duchene's muscular dystrophy is due to deletion of large parts of the X chromosome and the corresponding loss of several genes. Severity of this disease will depend on the regions lost and the associated genes. Multiplex PCR is used to amplify the possible 'hot spots' for deletion. More than 17 such different sites have been identified. A normal person will have all these sites and all relevant DNA bands will be visualized following PCR. Those affected will have fewer bands depending on the sites that have been deleted.

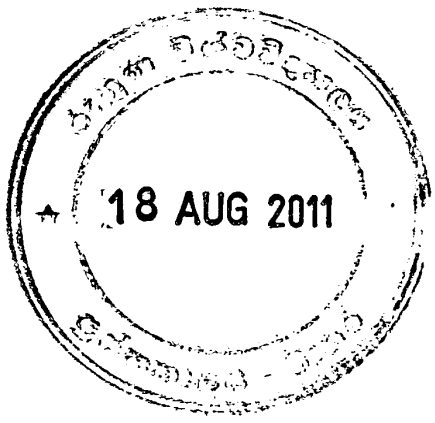
Human identification by DNA typing

DNA-based diagnostics have also become powerful tools in the identification of human specimens. All such methods are based on the principal that there are DNA regions (loci) in the human genome that vary between individuals (polymorphic loci), exhibiting a number of alleles in the population for a given such locus. The method of DNA typing used in our laboratory, the analysis of the highly polymorphic Short Tandem Repeat regions (STR loci) present in the human genome, is currently the most accepted method of DNA typing in the world. The alleles of a given STR locus differ in their lengths depending on the number of tandem repeats present in each allele. PCR amplification of STR loci and polyacrylamide gel electrophoresis of PCR products enable the determination of the alleles of a person for each locus tested. Analysis of a number of (9-12) STR loci generates an allelic DNA banding pattern (DNA profile), which is specific to an individual. The STR method of DNA typing enables the typing of minute amounts of DNA including partially degraded DNA, identification of sex, and positive identification of samples with a discrimination power of over 1 in a trillion. In criminal investigations, identification of biological samples by DNA typing has already proved useful to help convict criminals and to prove the innocence of falsely accused persons.

The same technique is also widely applied for the establishment of family relationships, including paternity. We have performed DNA typing for the state prosecution for criminal cases of sexual abuse, murder, child abuse and incest and also for civil cases of child maintenance and divorce. We have also provided DNA typing services for other countries (UK, USA, Canada, Sweden, Australia, India, Maldives) to verify family relationships with regard to child adoption, for testamentary and immigration purposes.

Limitations of molecular diagnostics

The need for expensive equipment and consumables, special laboratories and highly skilled staff make all PCR-based tests more expensive than conventional diagnostic methods. The accuracy and reproducibility of PCR depends on the technical experience and experience of the operator. PCR assays may be inhibited by substances present in the clinical specimen giving 'false negative' results, and 'false positive' results may arise due to PCR contamination. Hence, PCR laboratories must follow stringent quality control measures, and adhere to international standards. Despite the challenges of having to maintain high standards, I am proud to say that Genetech has moved forward untiringly towards realizing it's vision of making modern biotechnology accessible to the people of Sri Lanka.



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