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BioTech - 2016

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Colombo, Sri Lanka

Committee of the BioTech- 2016

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MESSAGE FROM PROF. ATTA-UR-RAHMAN, FRS KEYNOTE SPEAKER - BioTech 2016

Biotechnology has emerged in recent years as a powerful medium for socio-economic development. A large number of biotechnologies are being developed which include recombinant vaccines, vaccines in drug delivery, molecular diagnostics, bioremediation, sequencing of genomes, bioinformatics development, genetically modified crops, recombinant therapeutic proteins etc. Multibillion dollar industries are being developed in many of these fields and countries such as Cuba, which are investing massively in such technologies, have begun to benefit in a major way.

The three major players in the development of a knowledge economy are universities, industry and the government. All three thrive on the extent of merit-based competiveness that should be in-built into the systems and on the efficiency of interaction among all three players. The development of a knowledge economy requires a thorough understanding of the dynamic interplay between research, invention, innovation, and economic growth. Such an understanding allows them to be modulated according to national needs and challenges.

The global trends in manufacturing and exports over the last 3 decades clearly show a sharp rise in high and medium technology goods and a sharp consistent decline in low technology goods and in natural resources. The big money lies in high technology and countries that have realized this have invested massively in knowledge-based economies leading to the manufacture and exports of electronics, pharmaceuticals, engineering goods, biotech products, computers, software, automobiles, aircraft, defense equipment, alternative energy products and many other such items requiring highly specialized skills and world class research centres. That is why the annual exports of a tiny country which has a population only of about 5 million and no significant natural resources, Singapore, are a stupendous US \$ 458 billion.

Pakistan made remarkable progress in science and higher education during the 9 years (2000-2008) when I was Federal Minister for Science & Technology as well as the Federal Minister responsible for Higher Education that resulted in over 1000% increase in international publications in high impact journals and even greater increases in citations and PhD output. This laid the foundations of a strong knowledge economy.

I hope such issues as well as the exciting recent advances in biotechnology will be discussed in depth at the conference.

I wish Biotech-2016 all success.

Prof. Atta-Ur-Rahman. FRS

UNESCO Science Laureate/ Professor Emeritus, International Centre for Chemical & Biological Sciences, University of Karachi, Pakistan.



MESSAGE FROM PROF. SAMPATH AMARATUNGE

It is with great pleasure I warmly welcome all participants to the First Annual International Conference on Bioscience and Biotechnology 2016 on behalf of University of Sri Jayewardenepura, the hosting partner of the Conference which is organized by The International Institute of Knowledge Management (TIIKM) during 12-13 January at Colombo, Sri Lanka under the theme of "Molecular Life Sciences for the Development in the 21st Century".

Bioscience and Biotechnology, in both developed and developing countries are increasingly important fields in the modern era. The innovations and researches in this field are already impacting the society positively and hence we could expect more innovations that will contribute to the sustainability of human beings in this century. Main conference tracks being Bioprocess and Biological Engineering, Agriculture and Food Biotechnology, Bionanotechnology, Biopharmaceutics and Medical Biotechnology, and Biodiversity and Natural products. I have no doubt that this conference can promote such developments and innovations in the field of Bioscience and Biotechnology in this country and at the global level.

I wish the Conference every success and wish to see the knowledge and expertise derived from the 1st Conference will go a long way enriching the fields of Bioscience and Biotechnology and thereby contribute to the developmental activities both local and abroad.

Prof. Sampath AmaratungeVice Chancellor,
University of Sri Jayewardenepura,
Sri Lanka

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KEY NOTE SPEECH





[01]

THE WONDROUS WORLD OF INNOVATION

Atta-ur-Rahman

FRS

ABSTRACT

We live in a world where truth has become far stranger than fiction. Each day brings thousands of new discoveries, many of which are transforming our lives in a multitude of ways. Genes have been transferred from deep sea jelly fishes to orchids ---- the result are luminescent flowers that glow in the dark. Nanomedicines are being developed that are more effective than normal medicines. Super-fast gene sequencing under development should allow the entire human genome to be sequenced in minutes! Anti-ageing compounds have been discovered and when given to old mice, it made them younger! Stem cells promise to cure damaged organs and may change the manner in which medicine will be practiced tomorrow. In 2015 we have seen a research group at Guangzhou, China edit the genomes of human embryos using a new gene splicing technique: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) -Cas9 . It employs a strand of DNA that was discovered in 1987 to be part of a bacterial defense system. We can use this same mechanism to splice specific strands of our DNA - therefore CRISPR/Cas system is a way to edit our genome. It is cheap, fast and efficient way to accurately splice genes and provides us the tools to eliminate diseases and wipe out pathogens. The blind can, amazingly, today see using their tongue http://www.wicab.com/media/Wicab%20Press%20Release%203-19-2013.pdf. You can hang an elephant on a strand of "graphene" that is 150 times thinner than a human hair and the strand will not break since it is 200 times stronger than steel. Graphene is being employed to shuttle anti-cancer drugs to the sites of the tumours for more effective treatment. The Harry Potter's disappearing cloak is now a reality by the discovery of metamaterials. Anything covered with metamaterials just disappears since they have the ability to bend light. Bullet proof paper has been developed through application of nanotechnology. Objects can be moved by thought control and driverless cars are under development. Science today presents a myriad opportunities for research and exciting careers in many diverse fields. Some of these fascinating developments will be presented.





ORAL PRESENTATIONS





[01]

CYCLOTIDES DISTRIBUTION AND BIO-ACTIVITIES OF Geophila herbaceae

Sanjeevan Rajendran ¹, S.D.W. Mohotti ², Taj Muhammad ³, Robert Burman ⁴, Adam A. Strömstedt ⁵, E.D. de Silva ⁶, Ulf Göransson ⁷, C.M. Hettiarachchi ⁸, Sunithi Gunasekera ⁹

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ABSTRACT

Cyclotides are an unique class of ribosomal synthesized cysteine-rich mini protein. The structure consists of a head-to-tail cyclized backbone and three conserved disulfide bonds in a knotted arrangement. Due to these structural features cyclotides are ultra-stable and have attracted interest as peptide-based templates for drug design applications. The plant families Rubiaceae, Solanaceae, Fabaceae, Cucurbitaceae, and Violaceae are known to contain cyclotides.

The objective of this study is to investigate some selected plants of the family Rubiaceae for the presence of bioactive cyclotides. Initially, 17 plants of this family were screened for the presence of cyclotides using LC-MS techniques and *Geophila herbaceae* was selected for further study. Arial dried parts of *Geophila herbaceae* (140 g) was extracted with 60% aqueous methanol to yield 22 g of extract. LC-MS analysis of this extract showed the presence of about 15 different types cyclotides. Five of the cyclotides present in the mass range 2967.36+ Da to 3116.42+ Da were purified and sequenced using LC-MS-MS and were tested against microorganisms *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Out of the 5 cyclotides, 4 were inactive against all microorganisms tested at 100 μg/ml. The remaining cyclotide was active against all microorganisms at below the concentration 100 μg/ml. The cyclotides were also screened for cytotoxicity in the Fluorometric Microculture Cytotoxic Assay (FMCA) using Lymphoma U937 cells. All 5 cyclotides showed activity of 100% cell death in the range of 12.5-50 μg/ml. This study showed that *Geophila herbaceae* is a good source of bio-active cyclotides.

Keywords: Cyclotides, antimicrobial, cytotoxic activity.



[02]

VARIATION IN TOTAL POLYPHENOL CONTENT, ANTIOXIDANT POTENTIAL, THEAFLAVIN AND THEARUBIGINS CONTENT OF BOPF GRADE SRI LANKAN BLACK TEAS (Camellia sinensis L.) OF DIFFERENT CLIMATIC ELEVATIONS AND BOPF GRADE COMMERCIALLY AVAILABLE BLACK TEAS IN SRI LANKA.

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ABSTRACT

Tea is the most common nonalcoholic beverage in the world next to the water. Its popularity is attributed to its sensory properties, stimulating effects and potential health benefits. Black tea is the most popular tea among three major types of tea namely green, oolong and black tea. Unblended Sri Lankan black teas are classified as high grown, medium grown and low grown based on their geographical origin. In the quality control purposes, it is an added advantage if suitable chemical data that are helpful to distinguish between different teas are available. However some studies have been reported regarding the chemical parameters of black teas from different agro climatic elevations of Sri Lanka, such details like purity, cultivar and the particle size of teas have not been specified. And also there is no systematic screening has been reported for black tea commercialized in Sri Lankan market. So the main objective of this study was to analyze the total polyphenol content, antioxidant activity & total thearubigins & theaflavins content in unblended Sri Lankan black teas collected from factories representing different tea growing regions. And also to analyze the same biochemical parameters for the black teas that are commercialized in Sri Lankan market. During the month of April 2012, BOPF grade fermented black tea samples produced by TRI 2025 were collected from twelve tea plantations of different agro climatic elevations. All the teas were processed according to the orthodox-rotor vane method. Ten commercialized samples of different brands were also analyzed. Total phenolic content (ISO 14502-1 method), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, TF (flavognost method) and TR content were determined for each sample. Mid grown tea showed highest mean values in TPP content (15.9454 \pm 2.5778 % GAE), TF content (22.2200 \pm 3.9575 μ mol/g) and TR content (14.6031 \pm 1.8970 %). The antioxidant activity was well correlated with the total polyphenol content (P < 0.05) and thearubigins contents (P < 0.05). Lowest mean EC50 value (49.6791 ± 5.9475 µg/ml) was obtained by mid grown tea samples, indicating highest antioxidant activity among others. Rilagala plantation showed the maximum values in TPP content, antioxidant activity, TF and TR contents among others.

Keywords: black tea; antioxidant activity; total polyphenolic content; theaflavin; thearubigin



[03]

NERIUM OLEANDER LEAF EXTRACT MEDIATED SYNTHESIS OF NICKEL NANOPARTICLES AND ITS POTENTIAL APPLICATION

Ch Venkatanarasimha Rao ¹, Animes Kumar Golder ²

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ABSTRACT

In the past decades, nanotechnology is moving towards the maturity as a well-defined field of research and development. The bio-mediated technique for the synthesis of metal nanoparticles is easy to scale up for the large scale production in a cost-effective manner. The plant extract is consisting of active functional compounds that play a key role in the fabrication of process and, also prevents agglomeration of the particles. Nickel is cheaper than the many transition metals, and Ni nanoparticles (NiNPs) also could decompose the organic contaminants in a short reaction time. This work aims at the biosynthesis of NiNPs using NiSO4 as the metal precursor and aqueous leaf extracts of Nerium oleander as the reduction media. NiNPs were characterized using Fourier transfer infrared spectroscopy, Xray diffraction, Field emission scanning electron microscopy (FESEM), dynamic and electrophoretic light scatterings. The reduction kinetics of Ni ions into NiNPs was monitored through UV-Vis and Atomic absorption spectroscopies. The color of NiNPs solution was indicative of the size of the nanoparticles due to the surface plasmon resonance (SPR) band that could be speculated from the UV-Vis spectra. The FESEM image revealed the nearly spherical nature of NiNPs formed with the size distribution between 20 and 80 nm with an average size of 51 nm. Zeta-potential at varying pH was cognitive to NiNPs stability in the colloidal form. The activity of NiNPs was tested for Brilliant Green (BG) dye decolourization from the aqueous solution.

Keywords: Nickel nanoparticle, Biosynthesis, Colloidal stability, Dye Decolourization



[04]

QSAR STUDY: PREDICTION OF NATURAL THERAPEUTIC COMPOUNDS FOR MENINGITIS

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Karachi. Pakistan

ABSTRACT

Meningitis is a prevalent central nervous system disease which is characterized by acute inflammation of the membranes covering the brain and spinal cord. It is caused by bacteria, viruses and other microorganisms that gain entry into the cerebrospinal fluid. The growing resistance of various microorganisms to conventional drugs and the additional advantages offered by the drug-like natural compounds over them has resulted in an increased interest in this area of natural drug discovery. In this present Quantitative Structural Activity Relationship (QSAR) study, 6000 natural medicinal compounds (inactive/testing compounds) have been selected to compare the activity of these compounds with that of 49 active compounds (training compounds) used for the treatment of meningitis. All the compounds have been converted into symbols and numerals which show the physicochemical properties of molecules i.e. molecular descriptors. A random forest model is created which screened testing data set against training data set. It assigns RF score >0.5 to the predicted active compounds. As a result, 306 compounds out of 6000 natural compounds have been predicted to be active. Further analysis on these predicted compounds lead to the conclusion that many compounds could be lead candidates for therapeutics use and drug discovery in the treatment of meningitis.

Keywords: Meningitis, Drug discovery, Natural compounds Molecular Descriptors, QSAR.



[05]

ESTABLISHMENT OF A MOLECULAR BASED METHOD FOR THE IDENTIFICATION OF SKIPJACK TUNA (Katsuwonus pelamis) IN LARGE SCALE FISH PROCESSING INDUSTRY

Perera D.R.C ¹, Rodrigo W.W.P ², Athapaththu A.M.M.H ³, Gunathilaka P.A.D.H.N ⁴

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ABSTRACT

Skipjack tuna (Katsuwonus pelamis) is most often sold as canned light tuna and is the most common species found in tuna cans. In Sri Lanka differentiation of tuna species prior to processing is achieved through morphological identification, which is not a reliable method. Since the quality and market value of tuna products differ from species to species, a fraudulent replacement of valuable species with less valuable ones may occur. This has become a major limitation in fishery industry in order to reach products to the international market. Therefore, the objective of the current study was to establish a molecular based diagnostic method to differentiate skipjack tuna from other tuna species commonly found in Sri Lanka. Genomic DNA of skipjack tuna (*K. pelamis*), yellowfin tuna (*Thunnus albacares*) and bigeye tuna (Thunnus obesus) were extracted from the muscle tissues. Amplification of DNA from tuna samples were carried using genus specific primers which flank at 558 bp region of Cytochrome b gene. The amplified DNA products of tuna species were digested with ScaI restriction enzyme. The pattern restriction fragments evidence that products having band sizes of 215 bp and 343 bp were detected only from T. albacares (n= 8) and T. obesus (n= 8) while, K. pelamis (n= 8) was remained as an indigestive product (558 bp). Therefore, this can be used to differentiate K. pelamis from the other tuna species which are commonly found in Sri Lanka.

Keywords: Tuna species, DNA extraction, polymerase chain reaction, restriction enzyme digestion



[06]

ADEQUATE LEAST NUTRITIONAL SUPPLEMENTATION OF COCONUT WATER COULD LEAD TOWARDS DEVELOPMENT OF A SUBSTITUENT FOR ORAL REHYDRATION FLUID COMPLYING WITH WHO STANDARDS.

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ABSTRACT

In spite of being a nutrient and electrolyte rich fluid, coconut water is considered mostly as a waste in Sri Lanka. Acute diarrheal diseases are one of the leading causes of mortality in infants and young children in many developing countries which can be easily prevented by introduction of an electrolyte rich rehydration fluid. The major objective of this research was to analyze the feasibility of using coconut water as a rehydration fluid. Four areas from which coconuts enter in to a randomly selected market were selected for sample collection. Coconuts in three maturity states were considered from each area. Several rehydration biochemical properties were analyzed for each sample. Glucose content was analyzed using glucose oxidase-peroxidase method. Sodium, Potassium, Magnesium and Calcium contents were analysed using Flame Atomic Absorption Spectrophotometry (FAAS). Chloride content was determined using an Ion Selective Electrode. Osmolality of the samples was analysed using an Advanced Micro freezing point depression Osmometer. Anti-oxidant contents were analyzed using DPPH method. Each property was compared with WHO standards for the Production of new Oral Rehydration Salts. Potassium content and the Osmolality of all the samples were higher than the WHO standard levels and all the other properties were below the accepted levels. However samples contained considerable amounts of Calcium, Magnesium and Antioxidants. Based on this pilot study it can be concluded that only minimal supplementation is required towards development of an oral rehydration solution using coconut water. Further studies are ongoing aiming at ultimate value addition as well as waste management.

Keywords: coconut, rehydration, electrolytes



[07]

IN VITRO MICROPROPAGATION OF Gyrinops walla (GAERTH.) USING LEAF DISC EXPLANTS

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ABSTRACT

Gyrinops walla (Thymelaeaceae) is a slender tree which grows in wet zone regions in Sri Lanka. Potentiality of production of resinous natural product, agarwood, which used in perfumery and medicinal manufactures, grant a great demand to this species in worldwide. In vitro micropropagation technique may be a practicable solution to provide healthy planting materials in commercial scale, in order to overcome the problems caused to the natural population due to overexploitation. Thus, using leaf disc explant, possibilities of mass propagation through in vitro techniques was explored in in vitro study. Explants were surface sterilized using 0.2% carbendazim, 10% sodium hypochlorite and 70% ethanol each followed by two successive washings in sterile distilled water. Murashige and Skoog (MS) basal medium supplemented with varied concentrations of different plant growth regulators, 6benzylaminopurine (BA), 2,4-dichlorophenoxyacetic acid (2,4-D), Kinetin (Kin) and naphthaleneacetic acid (NAA) were tested for induction of calli from leaf discs. Out of different growth regulator combinations, rapid callus growth was observed in MS medium supplemented with 1.0 mg/L BA and 3.0 mg/L NAA within six weeks. Calli obtained were yellowish compact and callus induction was mainly observed along the mid-rib. Calli obtain could either be used for mass propagation, through indirect organogenesis or for establishment of plant cell culture to obtain secondary metabolites. From the results obtain, it could be concluded that callus initiation is feasible through leaf disc explant.

Keywords: in vitro micropropagation, callus formation, plant growth regulators, leaf disc explant



[08]

EFFECT OF CADMIUM ON MINERAL ELEMENTS CONTENT IN RICE (Oryza sativa L.) GRAIN

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ABSTRACT

The use of phosphate fertilizers plays a significant role in cadmium (Cd) accumulation in the soil (Grant et al., 2010; Tirado & Allsop, 2012). In this regards the purpose of our work was the study the effect of Cd on the content of mineral elements in the grain of rice varieties. Plants were grown in pots on soil, containing 2 mMol/kg of CdSO4. Determination of mineral elements performed by ICP-MS - Agilent 7500.

Cadmium was not found in rice grain. The content of mineral elements in grain of rice varieties is decreased in the following order (% of control): Mg - Bakanaskyi (98) > Madina (92) > Chapsari (86) > Barakat (84); Mn - Bakanaskyi (75) > Madina (72) = Chapsari (72) > Barakat (64); Zn - Madina (93) > Barakat (88) > Chapsari (87) = Bakanaskyi (87); Fe - Madina (89) > Bakanaskyi (72) > Chapsari (57) > Barakat (48); Cu - Madina (91) > Chapsari (90) > Bakanaskyi (88) > Barakat (85).

Cadmium decreased the content of necessary mineral elements in rice grain. Cadmium in the least degree reduced the content of mineral elements in Madina variety, in the greatest degree - in Barakat and Chapsari cvs.

Keywords: rice, cadmium, mineral elements



[09]

ANTHER RESPONSE IN INDICA RICE IS AFFECTED BY THE GROWING SEASON AND INORGANIC NITROGEN IN THE PRE-CULTURE MEDIUM

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ABSTRACT

Success of anther culture in rice is affected by genetic and non-genetic factors. This study investigated the effect of non-genetic factors on *in vitro* anther response in indica rice. In Sri Lanka, Yala and Maha seasons provide vastly different climate conditions for rice cultivation. Six anther culture responsive rice varieties (At 303, Bg 358, Bw 272-6B, Dahanala, Herath Banda, Dikwee) were raised in a Randomized Complete Block Design (RCBD) at the Regional Rice Research and Development Centre, Bombuwela, over the two rice growing seasons Maha (2013/14) and Yala (2014). From each variety, booting stage panicles were harvested, cold pre-treated and excised anthers were cultured on N₆ medium for callus induction. Anther response varied between varieties and for each variety a significantly higher response (p≤0.05) was observed in anthers cultured from plants grown in the Yala season than in the Maha season. The highest improvement was seen in variety Dahanala in which the anther response increased from 9.97% in Maha to 16.87% in Yala. Using rice variety At 303 the effect of short duration (3 day) pre-culture of anthers on N₆ medium having different inorganic nitrogen sources, NO₃ and/or NH₄ on anther response was examined. Callus induction frequency increased significantly (p < 0.05) with complete withdrawal of (NH4)₂SO₄ from the standard N₆ medium (10.38%) compared to the control (3.17%). Withdrawal of half the concentration of (NH4)₂SO₄ from the standard N₆ medium also recorded a fairly high callus induction response (5.39%). Thus, abiotic stresses applied to donor plants, as well as on anthers prior to culture on callus induction media, significantly improve anther response in indica rice.

Keywords: anther culture, indica rice, growing season, Nitrogen, pre-treatment



[10]

FIFTY PERCENT REPLACEMENT OF NITROGEN FERTILIZER RECOMMENDATION FOR RICE BY A BIOFILM OF A DIAZOTRPH AND A RHIZOSPHERE FUNGUS

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ABSTRACT

A biofilm was formed between the diazotroph Azorhizobium caulinodans and rice rhizosphere fungi Aspergillus spp. and was named Azorhizobium-Aspergillus biofilm (AAB). AAB colonizes rice roots and colonization is enhanced in the presence of flavonoid naringenin (AAB/Nar). This study investigates the percentage of recommended urea fertilizer levels that can be replaced by the developed biofilm (AAB) and naringenin combination. A pot experiment of rice with the treatments 100% ¹⁵N labelled Urea recommendation with no AAB/Nar (Control), 75% of ¹⁵N urea recommendation + AAB/Nar combination (75%) $U^{AAB/Nar}$), 50% $U^{AAB/Nar}$, and 25% $U^{AAB/Nar}$ was conducted for evaluating rice root colonization, nitrogen fixation (NFIX) and nitrogen yield (NYIELD) in 60 and 105 day old rice plants. A. caulinodans was labelled with a green fluorescent protein for in-vivo detection and the bacterial colonization was determined through green fluorescence intensities (Zen light, Zen 2012 software) & analysed by SAS version 9. ¹⁵N isotopic analysis of the pooled shoot and grain samples was done at University of California Davis, Stable Isotopic laboratory. The 60 day old plants showed the highest root colonization, shoot NYIELD and NFIX in treatment $50\%\,U^{AAB/Nar}$. Plants treated with $50\%\,U^{AAB/Nar}$ and harvested on 105 days had the highest grain yield, NYIELD and NFIX. It can be concluded that 50% of the urea recommendation can be replaced by the developed biofilm/Naringenin combination, to obtain even a higher rice yield than that of 100% urea recommendation.

Keywords: Nitrogen fixation, Biofilm, Rice, ¹⁵N labelling, Azorhizobium caulinodans



[11]

SEPARATION OF CATECHINS FROM GREEN TEA LEAVES BY MEMBRANES BASED TECHNOLOGY

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ABSTRACT

This work describes the improved method for the production of mixture of flavonoid compounds like Catechin, Epicatechin, Epigallocatechin gallate and Epigallocatechin from camellia sinesis. After studying various production routes for these compounds, several difficulties are sorted out and more appropriate technique is adopted for the production of catechin compounds. Here, membranes of ultra-filtration range having Molecular weight cut of (MWCO) 500 and 400 are prepared and used to separate these valuable components from crude mixture of extract prepared from leaves of camellia plant. The entire extraction process is carried without using organic solvents. Extract so prepared passed through cross flow as well as batch cell membrane to get the desired mixture. The experiments are carried out at various transmembrane pressures and cross flow velocity to study the extent of flux and purity of the product. The permeate is analyzed by using HPLC to judge the quality of the product. The final product is obtained by decaffeination of permeate followed by fridge drying. The final product is analyzed by using HPLC to judge the quality which is found to be above 70 %. This 70 % purity product is then further blended with various ingredients to produce tablets by compression moulding. Pure caffeine is produced in powder form. The economic analysis shows that the said process is more economical then the existing solvent passed process.

Keywords: Catechin, membrane, solvent, caffeine, green tea



[12]

PRODUCTION OF REDUCING SUGARS FROM SUGARCANE BAGASSE BY SUBCRITICAL WATER HYDROLYSIS

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ABSTRACT

Lignocellulosic biomass remains promising renewable feedstocks for the production of biofuels due to its abundance and ecofriendly nature. Sugarcane bagasse containing holocellulose of 62.93 wt% (40.15 wt% cellulose and 22.78 wt% hemicellulose) and lignin of 20.81 wt% is a potential feedstock for bioenergy production and was used for the production of reducing sugars using subcritical water (SCW) treatment in a batch reactor system. The effect of process parameters such as temperature (140-220 °C) and reaction time (5-40 min) on the release of total reducing sugar (TRS) was investigated. The production of TRS was increased with temperature from 140-180°C and thereafter decreased. Maximum TRS of 274.29 mg/g was obtained at 180°C and 30 min. The formation of sugar inhibitors (5-HMF or Furfural) gradually increase with an increase in the temperature. Maximum total inhibitors (TI) produced was 50.86 mg/g at 220°C and 40 min hydrolysis time, which includes 46.29 mg/g of 5-HMF and 4.57 mg/g of furfural. The crystallinity index (CI) of the SCW treated bagasse sample obtained in X-ray diffraction analysis was found to be increased with reaction temperature indicating disruption of lignin structure and release of pentose sugars thereby exposing the crystalline structure of cellulose, but further increase in temperature decreased the CI abruptly due to the conversion of cellulose to glucose. The percentage of CI was 54.03% and 63.32% at 140 and 160°C. At 180°C the CI % decreased to 59.96% which further decreased to 34.77% at 220°C thereby altering the biomass to relatively amorphous.

Keywords: subcritical water, bagasse, hydrolysis, sugarcane bagasse, reducing sugars, Inhibitors, Crystallinity.



[13]

DEVELOPMENT OF BIOCOMPOSITES FROM FOREST PRODUCTS: SUGAR PALM (Arenga pinnata merr.) BASED COMPOSITES

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ABSTRACT

Forest products are important sources of income of developing countries like Malaysia and research on utilizing them had been intensified. One of the forest products that is investigated at Universiti Putra Malaysia is sugar palm (*Arenga pinnate merr.*). Biocomposites made from sugar palm fibres and biopolymers had been developed using different types of plasticizers. In addition, sugar palm fibre alone can be a good raw materials for various domestic products like broom, rope, roof, headgears, just to name a few. The most recent work from sugar palm biocomposites was devoted to fabricating automotive component, i.e. anti-roll bar from hybrid glass/sugar palm fibre filled polyurethane composites. Materials characterization was performed and conceptual design of automotive anti-roll bar was developed and refined. Design analysis was performed using finite element analysis, ABACUS. Life cycle analysis was performed to study the sustainability aspect of the research. Fabrication process was performed using resin transfer moulding process.

Keywords: forest products, biocomposites, sugar palm, characterization, product development



[14]

Zingiber officinale Roscoe: SOME PROMISING INDIGENOUS VARIETIES FOR COMMERCIAL APPLICATION

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ABSTRACT

North eastern region of India is considered to be one of the richest biodiversity hotspots in the world. This region is treasured for one of the best qualities of fruits and vegetables, considered among the most important commercially. Known for its characteristic flavor and a wide range of health benefits, ginger is the main cash crop here and this region accounts for over seventy percent of India's total production, making the country's largest producer in the world. Surprisingly, this region houses some very potent indigenous accessions which are qualitatively multifold better than their commercial counterparts. Unfortunately lack of processing facilities account for their post-harvest losses to the tune of upto 95%, hence their use is limited only in their specific areas of origin and in folk medicine to some extent. This led us to undertake a systematic collection of indigenous varieties endemic to North East India for investigation of various characteristic quality parameters and compare them with the high value commercial varieties. Preliminary results show that Nagaland and Arunachal Pradesh varieties harvested at 6 months maturity level showed overall 2-3 times higher DPPH and ABTS radical scavenging activities than the commercial varieties and HPLC analysis showed percent 6-gingerol content of dry ginger powder 3 - 4 times higher than the commercial varieties like Karbi Anglong, Maran etc. These results could be utilized for commercial use in food and pharma industry, generating huge rural livelihood and hence reducing the post-harvest losses, putting these varieties to mainstream market.

Keywords: biodiversity hotspots, post-harvest losses, radical scavenging activity, DPPH, ABTS,% 6-gingerol content.



[15]

SCREENING OF SPRING WHEAT VARIETIES RESISTANT TO SOIL POLLUTION WITH HEAVY METALS TO CREATE ECOLOGICALLY PURE PRODUCTION

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ABSTRACT

The aim of this research work is to identify germplasm of the wheat that is resistant to heavy metals (zinc, cadmium) and which is priority in the East Kazakhstan, for identification of donors for selection on metal resistance and promising forms for production.

Experiments were performed on 10-days-old sprouts of different wheat genotypes, which were grown on nutrient mixtures containing Cd ions in concentration 40 mg/l (CdSO4) or Zn in concentration 400 mg/l (ZnSO4). The measurement of biometric parameters carried out by conventional methods. Atomic absorption spectrophotometry has been used in order to determine the concentration of heavy metals (cadmium and zinc). In addition, growth parameters and tolerance index has been defined.

Based on the results received in 2015 the following conclusion was drawn: throughout the experiment "Lyazzat" and "Samal" spring wheat varieties could be considered as the tolerance genotypes to zinc translocation to the over ground organs. In case of cadmium translocation most steady wheat cultivars were "Lyazzat", "Omskaya18" and "Altai", while the most sensitive was "Eritrospermum" wheat variety. Permeability of the membranes for electrolytes of steadier cultivars such as "Altai" has been changed to a lesser extent compare to more sensitive one like "Eritrospermum". The result has showed that this fact in both cases due to effect of cadmium as well as zinc. This fact testifies that stability of plants in general can be caused by resistance of their cellular membranes to the action of stressor. Research has revealed tolerance and sensitive genotypes.

Keywords: heavy metals, wheat, growth parameters, accumulation of metals, membrane permeability, sensitive and tolerance genotypes.



[16]

MOLECULAR CHARACTERIZATION OF COAGULASE GENES OF Staphylococcus aureus ISOLATED FROM MASTITIC RIVER BUFFALOES

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ABSTRACT

Mastitis is a major dairy herd problem mainly caused by *Staphylococcus aureus*. The *Staphylococcus aureus* isolated strains from mastitic milk samples were subjected to molecular characterization of coa gene. The DNA was extracted by using DNA purification Kit. The PCR reaction of representative samples was run and sequencing was performed in Australian Genomic Research Facility (AGRF). A phylogenetic tree (using MEGA6.1 software package) was constructed which revealed that the coa gene of *S. aureus* strains, could be grouped in two clades which were closely related to S. aureus isolates from Japan, India and Taiwan, while S. aureus isolates from Germany, UK and USA were distantly related. These results indicated the genetic relatedness of Pakistani isolates with other reported isolates from different parts of the world. All the Pakistani strains of coagulase gene were 99.98-100% similar while their divergence was 0.02%. The Pakistani strains were 99.9% similar and 0.07% divergent from Indian, Japanese, Taiwan, UK and German reported strains. These findings will also be helpful in future for designing suitable mastitis control strategies in the country.

Keywords: Buffalo, Mastitis, *Staphylococcus aureus*, Coagulase gene.



[17]

IN VITRO AND IN VIVO EFFICACY OF FRACTIONS OF Sida cordifolia L. AGAINST HERPES SIMPLEX VIRUS TYPE I INFECTION

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ABSTRACT

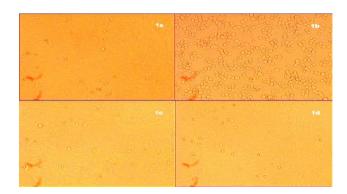
Introduction: There is an increasing need for substances with antiviral activity since the treatment of viral infections with the available antiviral drugs often leads to the problem of viral resistance. The fractions of toluene and hydroalcoholic extracts of *Sida cordifolia* L. were investigated for potential antiviral effect against HSV-I *in vitro* and *in vivo* and efforts had also been taken to study the underlying mode of action.

Materials & Methods: The extracts and factions were screened for their cytotoxicity against Vero cell lines by MTT and SRB assays. The antiviral effect was tested in infected Vero cells by MTT antiviral, plaque reduction assay and mechanism based studies were carried out by virus adsorption, penetration and replication assay. The therapeutic efficacies were characterized using a cutaneous HSV-1 infection in mice.

Results: The alkaloid fraction (FHA) of hydro alcoholic and phytosterols of (FP) of toluene extract reduced viral infectivity and produced cell protection by $96 \pm 1.02\%$ and $95 \pm 1.22\%$ against HSV-I (figure 1) when challenged with highest virus challenge dose (100 TCID₅₀). All the extracts showed dose dependent activity with viral strain. The FP fraction at a dose of 750 mg/kg per day significantly delayed the development of skin lesions (P<0.05), prolonged the mean survival times and reduced the mortality of HSV-I wild type 7401 virus infected mice as compared with 0.5% DMSO in distilled water (P<0.001).

Conclusion: Present investigation finding reveal that the toluene fraction of *Sida cordifolia* L. have good antiviral potential and may be possible candidate for the development of anti HSV-I agents.

Keywords: Cytotoxicity, HSV-I, MTT assay, *Sida cordifolia L.*, SRB ass



a) Vero Cell Control. b) HSV-I virus control. c) Cell culture treated with the extract after virus challenge, FP fraction at 50 μg/ml. d) Standard drug- Acyclovir at 10 μg/ml

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[18]

IN VITRO ANTIBACTERIAL STUDY OF PEEL, PULP AND SEED KERNEL EXTRACTS OF Mangifera indica AGAINST SELECTED BACTERIAL PATHOGENIC STRAINS.

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ABSTRACT

Mango (Mangifera indica) is an important tropical fruit worldwide due to its flavor and health promoting activity. Mangoes available in dry zone area of Sri Lanka have high consumer demand due to unique flavor. The main aim of this study was to investigate antibacterial activity of the peel, pulp and seed kernel of Mango. In the present study, three mango cultivars, namely 'Willard', 'Karuthacolomban' and 'Vellaicolomban' were collected from Jaffna district in Sri Lanka during April to July, 2015 to carry out the extraction processes. Ethyl acetate extracts of flesh, peel and seed kernel of three mango varieties were tested for antibacterial activity using agar well diffusion method at 10 mg/mL against Escherichia coli [ATCC 25922], Staphylococcus aureus [ATCC 25923] and Bacillus subtilis [MTCC 121]. The results revealed that seed kernel of 'Karuthacolomban' and 'Willard' showed antibacterial activity against E. coli. Seed kernel extracts of 'Karuthacolomban', 'Willard' and 'Vellaicolomban' showed the antibacterial activity against S.aureus and B. subtilis. None of peel and pulp extracts inhibited the growth of E.coli, S. aureus and B. subtilis. The active extracts were next tested at 1.25, 2.50, 5 mg/mL. At 5 mg/mL concentration, seed kernel of 'Karuthacolomban' showed the antibacterial activity against E. coli and S.aureus. The above observation suggests that ethyl acetate extract of "Karuthacolomban' seed kernel shows the highest antibacterial activity against all the three tested microorganisms. These results demonstrate that seed kernel of mango extracts are capable of producing antibacterial substance against selected pathogenic bacteria.

Keywords: Mango, peel, seed, pulp, Antibacterial activity



[19]

ANALYSIS OF THE OCCURRENCE OF CIS-REGULATORY ELEMENTS IN THE FIRST INTRONS OF HUMAN GENES

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ABSTRACT

Gene expression is primarily regulated through the interaction between transcription factors and their cognate cis-regulatory elements found in the promoter region of a gene. However, research studies have revealed, that the first introns of certain human genes also play a substantial role during the gene expression. A recent study has shown that the majority of the first introns of human housekeeping and tissue specific genes harbor cis-regulatory elements, like CAAT, GC and TATA boxes. The objectives of this study are to investigate the occurrence of such elements in the first introns of human housekeeping and tissue specific genes and to evaluate the first intron sequence similarity between human and closely related species. Appropriate first intron sequences were extracted from the ensembl genome browser. The knuth morris pratt algorithm written in perl, was executed to obtain motif counts. Expected motif counts for a given sequence were calculated using perl scripts. Results show, the motif counts do not directly proportionate to first intron length. Moreover, the average CAAT box count is higher in first introns of tissue specific genes and more than 95% of first intron sequence conservation was observed between human and closely related species which is a novel finding. Conclusively, this study speculates that the occurrences of motifs are unlikely to occur by chance along the first introns and there may be a correlation between the higher CAAT box counts and tissue specificity. Moreover, first intron sequences between human and closely related species are very highly conserved during evolution.

Keywords – Gene expression, human genes, first introns, cis-regulatory elements



[20]

INVESTIGATION OF METHYL TETRAHYDRO-FOLATE REDUCTASE C677T POLYMORPHISM IN A SAMPLE OF PATIENTS IN A HOSPITAL BASED DIAGNOSTIC SETTING IN SRI LANKA.

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ABSTRACT

The present study was conducted to identify the prevalence of MTHFR gene mutation in a group of Sri Lankan patients seeking routine diagnostic testing for the C677T mutation through PCR followed by RFLP diagnostic methods. Human MTHFR gene codes for the production of MTHFR enzyme in humans which plays major role in homocysteine and folate metabolism in humans. The mutation in MTHFR gene at nucleotide position 677 has two possibilities: C (cytosine, which occurs in the wild type gene) or T (thymine, which occurs in the mutated gene). In C677T variant, modification from C to T gives rise to the replacement of alanine by valine residue in protein at 222 aa position. Eight serum samples were collected from the MTHFR mutation suspected patients. Genomic DNA extraction was done and extracted DNA was subjected to PCR amplification. PCR products were subjected to RFLP assay with overnight digestion. The substitution of "C" to "T" at 677 position of MTHFR gene creates a restriction site for the enzyme Hind111. RFLP products were subjected to gel electrophoresis and further analysis. The results obtained shows that, of the 8 samples tested, 2 had 677CT genotype, 1 had 677TT genotype whereas the remaining 5 had 677CC wild genotype Homozygous leads to lower **MTHFR** activity 677TT hyperhomocysteinaemia, which can cause defects in DNA repair. However, as this is a small sample of the population, it is recommended to carry out studies with a large sample volume to ascertain the prevalence of this mutation in the community.

Keywords - Methylene tetrahydrofolate reductase (MTHFR), MTHFR gene mutation (C677T variant), Genotype, Homocysteine, Folate, Restriction fragment length polymorphisms (RFLP), Polymerase chain reaction (PCR), DNA extraction, Restriction site, Hind111, Gel electrophoresis, Hyperhomocysteinaemia, DNA repair.



[21]

REAL TIME PCR ASSAY FOR THE DIFFERENTIATION OF MYCOBACTERIAL SPECIES IN BRONCHIAL WASHINGS

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ABSTRACT

Pulmonary infections caused by Nontuberculous Mycobacteria (NTM) species has to be carefully interpreted due to their ubiquitous nature. NTM infections are more common than before in non-immunosuppressed hosts. Real-time PCR designed for Mycobacterium species, allows precise identification through melting point analysis. This study was designed for identification of Mycobacterium species present in bronchial washings. Ethical clearance was obtained from the Post-Graduate Institute of Science, University of Peradeniya. Bronchial washings (n=150) were collected from patients, suspected of having pulmonary diseases, attending the General Hospital Kandy. The samples were processed according to modified Petroff's method and inoculated onto Löwenstein- Jensen medium. Culture positives were subjected to Ziehl-Neelsen(ZN) staining, DNA were extracted from AFB isolates using the standard CTAB (N Cetyl-N, N, N-trimethyl ammonium bromide) method.SYBR green mediated real-time PCR assay was conducted to identify rapid and slow growers in two parallel reactions. Primers specific for Mycobacterium genus, Mycobacterium tuberculosis complex (MTC), M. avium complex (MAC), M. chelonae- M. abscessus group (MCAG) and M. fortuitum group (MFG) were used. Among the 26 AFB isolates 25 were found to be belonging to the Mycobacterium genus. Two MTC isolates and three MAC isolates were confirmed, following reaction I. Reaction II confirmed the presence of Mycobacterium genus and the presence of MCAG for two isolates. Application of SYBR green mediated real time PCR assay in clinical microbiology could improve the diagnostics due to the increased specificity. Moreover, it is a tool that can be used for the rapid detection of pathogenic NTM species.

Keywords: NTM, SYBR Green, Real-Time PCR, AFB



[22]

ANALYSIS OF BLOOD TRANSCRIPTOME IN PATIENTS WITH CHRONIC KIDNEY DISEASE OF UNCERTAIN AETIOLOGY

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ABSTRACT

Chronic kidney disease of uncertain aetiology (CKDu) is an increasing health problem in certain agricultural regions of the tropical world including Sri Lanka. The purpose of this study was to analyse blood transcriptome patterns of CKDu patients in comparison to healthy individuals, and to identify differentially expressed genes as possible biomarkers of CKDu. Briefly, total RNA was isolated from whole blood of Stage 2, 3 and 4 CKDu patients from a CKDu endemic region of Sri Lanka, Girandurukotte, and healthy individuals of a CKDu nonendemic region, Kandy. The RNA was pooled stage-wise and expression patterns were compared to that of pooled RNA of healthy individuals. The RNA was amplified, reverse transcribed and hybridized to Illumina HumanHT-12 v4 Expression BeadChip arrays and scanned with an Illumina Bead Array Reader Confocal Scanner. A total of 974, 1034, and 67 genes were differentially expressed (fold change ≥ 2 or ≤ 0.5) in Stages 2, 3 and 4 CKDu respectively. Of these, seven genes were identified that were commonly differentially expressed in the three different stages compared to the healthy group. The genes identified included those involved in hypertensive response (ADM), gap junction channel activity (GJB4) and infectious/immune response (IFIT1, PI3, DEFA1, HBZ, RN7SK). These genes have the potential to be used as diagnostic markers of CKDu as they are differentially regulated in the three studied stages of CKDu and can diagnose disease patients from the healthy individuals. Real time quantitative PCRs are currently being carried out to verify findings of the microarrays.

Keywords: Biomarkers, CKD, CKDu, gene expression analysis, peripheral whole blood, RT-qPCR



[23]

CONTROLLED RELEASE OF DOXORUBICIN FROM CHITOSAN AND CHITOSAN-ALGINATE NANOPARTICLES AND *IN* VITRO CYTOTOXICITY STUDIES ON MCF-7 CELL LINE

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ABSTRACT

The anthracycline antibiotic doxorubicin (DOX) is a drug used in treatment of different cancers including breast cancer. The clinical usage of doxorubicin has limitations since it is associated with increased risk of cardiotoxicity with high administrated dosage. We hypothesize that toxic side effects could be minimized with maximized efficiency by encapsulating DOX in a carrier system. In this study, main objective was to develop a new slow release carrier system for the anticancer drug DOX using biopolymeric nanoparticles and to compare the controlled release and the cytotoxicity with the free DOX. Two polymeric nanosystems; chitosan and chitosan-alginate nanoparticles were prepared using modified ionic gelation method to encapsulate the anticancer drug DOX. After characterizing the successful loading of DOX into nanoparticles, the in vitro cumulative release of DOX from nanoparticles was determined in distilled water and phosphate buffered saline solution (pH~7.4). In vitro cytotoxicity studies, apoptosis studies and cellular uptake studies were done on human breast cancer (MCF-7) cell line with DOX loaded nanoparticles and with free DOX. Both nanocarrier systems produced similar release profiles which showed an initial burst release phase followed by slow release phase. But higher cumulative release of DOX was obtained with chitosan-alginate nanoparticles. The in vitro cytotoxicity results showed the dose and time dependent behavior of DOX loaded nanoparticles while the free DOX showed only a dose dependent cytotoxicity. Moreover the IC50 values obtained for DOX loaded chitosan-alginate nanoparticles were lower than that of the DOX loaded chitosan nanoparticles. Overall results indicated that DOX loaded nanoparticles enhanced the efficacy of the DOX while releasing the DOX slowly from the nanoparticles with reported proapoptotic effects of DOX.

Keywords: slow release, doxorubicin, chitosan, alginate, cytotoxicity



[24]

TO IDENTIFY THE HUMAN ATAXIA TELANGIECTASIA MUTATED AND RAD3 RELATED (ATR) CORE PROMOTER SEQUENCE CRUCIAL FOR TRANSCRIPTIONAL REGULATION BY P²¹ ACTIVATED KINASE 1 (PAK1) IN CANCER CELLS

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ABSTRACT

P²¹ activated kinase 1 (Pak1), a major mitogen-responsive serine/threonine signaling kinase is a well-known regulator of cytoskeletal remodeling that contributes to tumor formation. Alterations in Pak1 expression has been documented in many type of cancers and its role in DNA damage response (DDR) was well reported. DNA damage response to UV involves the activation of Ataxia Telangiectasia mutated and Rad3 related (ATR) a serine/threonine kinase and its functional Dysregulation leads to increased tumourogenesis. Yet, the knowledge of ATR transcriptional regulation was feebly known. In our current study Pak1 overexpression and knockdown clones were generated in keratinocytes using viral transduction and observed a significant change in ATR expression with Pak1 confirmed by real-time quantitative RT-PCR and Western blot analysis. In addition Active Pak1 mutant is more efficient in ATR regulation indicating that ATR as the probable transcriptional target of Pak1. Further our studies with ATR promoter using luciferase reporter assays and by Deletion analysis we identified that 652bp fragment is the core promoter sequence being regulated by Pak1.In conclusion, our results specify for the first time, that Pak1 is able to regulate the ATR expression, considering ATR to be one of the possible target of Pak1 in DNA damage response.

Keywords: P²¹ activated kinase 1 (Pak1), Ataxia Telangiectasia mutated and Rad3 related (ATR), keratinocytes.



[25]

KIBRA: NEW FAMILY MEMBER OF ATAXIA TELANGIECTASIA MUTATED (ATM) KINASE

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ABSTRACT

KIBRA, originally identified as a protein expressed predominantly in KIDney and BRAin, has been shown to be highly expressed in breast cancer cells and plays an important role in growth and survival of breast cancer cells. Since KIBRA is widely expressed in breast cancer cells and because cell survival functions of KIBRA might counteract DNA-damage response. Our *in vitro*, in vivo data and from ATM over expression and ATM null model systems provide evidence that KIBRA is a physiological substrate of Ataxia Telangiectasia Mutated Kinase (ATM) - a master regulator of DNA Damage Response (DDR) pathway. We identified the site of phosphorylation by site directed mutagenesis, and further confirmed the same with motif specific antibody. Our work introduced KIBRA in ATM kinase family

Keywords: KIBRA, Ataxia Telangiectasia Mutated Kinase (ATM)



[26]

MOTOR PLASTICITY INDUCED BY INTERMITTENT THETA BURST CORTICAL ELECTRICAL STIMULATION IN RATS WITH TRAUMATIC BRAIN INJURY

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ABSTRACT

Traumatic brain injury (TBI) is a growing public health concern, with increasing needs for more rigorous measures to quantify changes in the brain post-injury. Electrophysiologic methods, and in particular, transcranial magnetic stimulation (TMS) or cortical electrical stimulation (CES), have been demonstrated to provide prognostic value in several neurological disorders. However, no study has been reported to quantify the electrophysiological changes by using CES method following TBI. This study investigated corticomotor excitability and inhibition in TBI rat model. Cortical excitability was studied in 7 TBI and in 8 normal rats using paired pulses CES. The parameters of testing included resting motor threshold (RMT), recruitment curve (REC) of motor evoked potential (MEP) and long-interval cortical inhibition (LICI) at long intervals (50, 100 and 200 ms). Furthermore, the changes of motor plasticity induced by intermittent theta burst stimulation (iTBS) were also tested in in normal and TBI animals. In results, the TBI group overall revealed a lower RMT and narrower recruitment curves compared to normal rats (p<0.05). The alterations in LICI were more pronounced in TBI rats (p<0.05). Also, iTBS-induced potentiation in MEP was reduced in TBI rats (p<0.05). In Conclusion, this study was the first to demonstrate differences in motor plasticity and intracortical inhibition in TBI animal model. Based on our results, brain injury may alter the neural activity in electrophysiological performance. Longitudinal studies in individuals with TBI would be valuable to identify this hypothesis further, which might provide prognostic biomarkers and suggest novel therapeutic strategies.

Keywords: cortical electrical stimulation, long-interval cortical inhibition, motor evoked potential, neural plasticity, theta burst stimulation, traumatic brain injury



[27]

INVERSE RELATIONSHIP OF STATIN USE WITH VITAMIN D DEFICIENCY IN A PAKISTANI POPULATION

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ABSTRACT

There is high prevalence of vitamin D deficiency in Type II diabetes mellitus in Pakistani population. Type II DM patients with dyslipidemia are generally prescribed statins to reduce the risk of cardiovascular events. The aim of this study was to investigate the effect of statins on vitamin D status of Pakistani type II DM patients and healthy adults. In a cross-sectional survey, 165 consecutive patients with type II DM (116 males and 49 females, age 22-70 years) and 165 gender-matched healthy adults were recruited with informed consent. A questionnaire was administered to find out whether they were statin users or non-users. Serum/plasma was analyzed for concentrations of 25(OH) vitamin D [25(OH)D] and related biomarkers using kit methods. Study participants were divided into vitamin D deficient [(25(OH)D <20 ng/mL)] and vitamin D non-deficient [(25(OH)D >20 ng/mL)] subjects. Binary logistic regression was used to evaluate association of statin use with vitamin D deficiency. Mean serum level of cholesterol was significantly higher in the vitamin D deficient group compared to non-deficient group (P=0.002). Vitamin D deficiency was significantly more common among males (P=0.025); while statin users had better vitamin D status compared to non-users (P=0.001). The odds ratio (OR) for the association of vitamin D deficiency was nearly one-half in statin users compared to non-users, [OR(95% CI) = 0.48(0.28-0.88); P < 0.01] when the model was adjusted for gender, cholesterol, albumin and phosphate. There is an inverse association between statin use and risk of vitamin D deficiency in Pakistani population of patients and controls.

Keywords: Statins, Vitamin D deficiency, Diabetes mellitus, Hypovitaminosis D, Pakistani population



[28]

COMPARATIVE PLASMA PROTEOME PROFILING REVEALS PUTATIVE BIOMARKERS OF CHRONIC MYELOID LEUKEMIA

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ABSTRACT

Deregulation of many signaling pathways is involved in the onset and progression of different forms of cancer including leukemia, a malignant hematological neoplasm that leads to abnormal proliferation of white blood cells in the bone marrow. During the course of leukemia, gradual amplification of mutational events at the genetic and/or epigenetic levels are believed to have major knock-on effect in altering and activating the expression of different mitogenic, anti-differentiating and anti-apoptotic modulators, which affect the plasma proteome profiles as well. Analysis of these altered protein profiles in patients and their healthy counterparts are likely to assist in keeping track of the concealed perturbations while expediting the search for novel biomarkers and therapeutic targets of the disease.

We have employed two-dimensional gel electrophoresis in conjunction with mass spectrometry-based analysis for the identification of biomarkers of chronic-phase chronic myeloid leukemia (CP-CML). Over 1300 plasma protein spots were subjected to analysis by MALDI-TOF mass spectrometry, which resulted in identification of 33 distinct proteins and their respective isoforms/subunits. Comparative analysis revealed differential expression of six proteins which were subsequently confirmed through ELISA with p-value <0.005. Amongst these candidates, three proteins were successfully validated both in the pre-treated and nilotinib-treated CP-CML cases, using quantitative ELISA (ANOVA p=0.000). Further, a curated pathway of differentially expressed proteins was built up, which seems to translate the probable association of marker proteins with the BCR-ABL-driven genomic instability. This, to our knowledge, is the first study from Pakistan that describes systematic plasma proteome profiling of local CP-CML patients.



[29]

IMPORTANCE OF EARLY DETECTION OF CABBAGE WHITE MOLD IN SRI LANKA

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ABSTRACT

Severe white mold infection on cabbage was detected in Pattipola, Ambewela and Seethaeliya in early 2014. No disease free field was observed. Farmers were unaware of the disease, pathogen survival, etiology, alternative hosts and on effective management practices. The aim of this study was to study the pathogen biology, genetic diversity and the mode of disease spread in Sri Lanka. Molecular and morphological characterization and Kotch's postulates confirmed that the pathogen causing cabbage head rot in Sri Lanka is, a necrotrophic fungal plant pathogen, Sclerotionia sclerotiorum. The pathogen produces survival structures, sclerotia, which can survive in soil for more than eight years. Nearly 100-200 sclerotia were found on un-harvested infected cabbage heads. A careful field observation found that there was at least 4 infected cabbage heads per infection foci. Upcountry vegetable farmers usually cultivate tomato, potato, lettuce, beat and beans as rotational crops. However, the pathogen can infect more than 400 plant species including all these economically important vegetables and the infected carrots and beans were already found. Mycelial Compatibility Grouping (MCG), a self, none-self recognition system, was done to infer the genetic diversity of the population. High MCG diversity was observed. Insecticide application due to the misdiagnosis of the disease, cabbage cull piles, crop rotation with alternative hosts and poor weed management were often practiced by these vegetable farmers. Therefore, early detection in the field is important since the emerging cabbage head rot pathogen can cause severe damage to other vegetable hosts in the region, the pathogen can produce more survival structures on cabbage cull piles contaminating the soil and due to the presence of high genetic diversity, the disease management will be challenging if neglected.

Keywords: Cabbage head rot, MCG, *Sclerotionia sclerotiorum*, disease management, sclerotia



[30]

IN-VITRO MULTIPLICATION OF GINGER (Zingiber officinale Rosc.) CULTIVARS -LOCAL, CHINESE AND RANGUN

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ABSTRACT

In Ginger, it is necessary to find an alternative method for production of disease free planting materials as the conventional propagation technique through rhizomes transmit many diseases. This study summarizes an efficient micropropagation method developed for Zingiber officinale cultivar, Local, Chinese and Rangun using sprouted bud explants from fresh rhizomes. Rhizome buds with the size of 0.5-1.0cm were surface sterilized with 30% Clorox for 30 minutes prior to culture and this treatment resulted 60%-70% healthy pure explants for all three cultivars. Murashige and Skoog (MS) (1962) medium has shown the best performance in culture establishment for three cultivars. Highest survival rate of the explants was observed in the Chinese while lowest was observed in Local. MS medium supplemented with 2mgl⁻¹ BA and 0.25mg l⁻¹ NAA was shown the best performance for initial multiplication of ginger buds (8.6 shoots per explants) in Local. Rangun and Chinese were shown the highest rate of multiplication (respectively 7.33 and 7.20 shoots per explants) in the MS supplemented with 4.0 mg l⁻¹ BAP and 0.25 mg l-1 NAA. Half strength of MS medium supplemented with 1.5 -2.0mg l⁻¹ NAA has shown induction of roots in ginger cultivars tested. Of the combination tested ½ strength MS with 1.50 mg l⁻¹ NAA elicited optimal rooting response in an average of 5.5 roots with 3.64 cm root length for Local. But the highest value of root length 4.95(cm) and 4.12(cm) were recorded in plantlets of Rangun and Chinese respectively in the half strength MS medium with 2.0mgl⁻¹NAA.

Keywords: Ginger, explants of sprouted buds, micropropagation, Zingiber officinal



[31]

DEVELOPMENT OF READY-TO-SERVE (RTS) FRUIT DRINKS WITHOUT ADDING PRESERVATIVES

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ABSTRACT

Microbial safety of commercially available RTS is achieved by adding higher levels of preservatives than recommended. Chemical preservatives at higher intake results in health problems. Hence this study was carried out to find out to produce RTS without adding chemical preservatives.

For level of acidity was selected and to protect their sensory properties the same Brit/Acid ratio was maintained. Both pineapple and mix fruit juices prepared using higher levels of acids were sensory evaluated for their suitability of consumption. Brit/Acid ratio 14.8/0.32 was a final to be significantly acceptable for both juices.

Microbial studies showed all the treatments were free of microbial growth even without added chemical preservatives. No microbial growth was observed in commercial Brit/Acid ratio. Hence higher acid level added treatments can commercially be recommended to produce RTS without significant changing of sensory properties. Hence preservatives added for the preservations of RTS can be eliminated by increasing acid level through maintaining Brit/Acid ratio.

Keywords: RTS, Preservation, Sugar/Acid



[32]

INTEGRATED USE OF BIOFERTILIZERS AND BIOCOATED CHEMICAL FERTILIZERS IN PAKISTAN

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ABSTRACT

As the world strives to reduce NPK fertilizer usage and increase the sustainability of its agriculture, it is facing a rapidly growing population, which will soon require more food than current agricultural methods can produce. In this scenario it is important to find means of improving yields that do not rely on the application of the chemicals alone. In view of persistent relatively high cost of fertilizer, the exploitation of integrated use of chemical fertilizers with biological fertilizers is an attractive strategy for developing sustainable agriculture production. Bio fertilizers coated chemical fertilizer (Bio active Urea) and Bio organic Phosphate (BOP) has been prepared and their effect on the cost and yield of the crop has been studied .In comparison chemical DAP and BOP, BOP gave 23-24% higher grain yield in wheat and maize and 15-40% higher biomass in wheat, maize and sorghum. In case of Bio active Urea the increase in yield was 18-20% as compared to normal Urea. The reduction in the cost of fertilizers was 40% in comparison to chemical fertilizers. The use of Bio Coated chemical fertilizers is currently gaining interest in Pakistan as cheap and safe alternate to chemical fertilizers without compromising the yield and quality of crops. During the last six months about 100,000 bags of bio coated urea has been utilized by the farmers. Results showed the significance of integrated use of Biofertilizers and chemical fertilizers. As the farmer field results are very promising it is obvious that this technology can also work well in Sri Lanka.

Keywords: Biofertilizers, Bio coated chemical fertilizers.



[33]

UTILIZATION OF RUBBER SEED FOR BIOFUEL AND VALUE-ADDED CHEMICALS SYNTHESIS

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ABSTRACT

Rubber seed (RS) is one of the promising non-edible oil seed for biofuel synthesis. In this work RS comprises of 40-48% shell and 52-60% kernel by weight of seed was studied for fuel and value-added chemicals production. RS oil (RSO) from kernel was extracted by soxhlet extraction using various organic solvents. The effects of process parameters such as extraction time (3-12h), kernel size range (0.5-3mm), ratio of kernel to solvent (0.03–0.09g/ml), and solvents (polar and non-polar) on extraction process were studied and optimized using response surface methodology (RSM). The optimum conditions for extraction which could produce 49.36wt% RSO yield were 0.08g/ml of kernel to hexane ratio and 1mm particle size at 8h extraction time. Further the extracted RSO was used for synthesis of methyl ester (biodiesel). Two steps procedure, esterification with catalyst H₂SO₄ followed by transesterification with Ba(OH)₂•8H₂Owas performed using ultrasonic technique. The effect of process parameters viz. catalyst loading, reaction time and molar ratio of methanol to RSO on esterification and transesterification reaction were studied. Maximum conversion RSO (~97%) was achieved at catalyst loading 5.38wt% with methanol to oil molar ratio 8.09:1 in 14min. Further, detailed thermogravimetricanalysis (TGA) and reactor pyrolysis of solid byproducts viz. shell and cake of the RS were conducted to study pyrolysis temperature range and for production of targeted liquid and solid products for fuel and chemical synthesis. Physico-chemical properties and composition of solid and liquid products were estimated to measure its suitability for biodiesel, chemical and fuel production.

Keywords: Solvent extraction, RSM, RSO, biodiesel, transesterification, TGA, pyrolysis



[34]

THE EFFICIENT RECOVERY OF POLYHYDROXYBUTYRATE BIOPOLYMER BY NON-TOXIC SOLVENTS

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ABSTRACT

Polyhydroxybutyrate (PHB) is a thermoplastic biopolymer that accumulates in wide range of microorganisms under stress conditions. In recent years, PHB have attracted increasing attention because it could be substitutes for conventional plastics. The recovery of PHB granules from bacterial cytoplasm significantly increases total processing costs. Efficient and economical recovery of PHB from cells is required for it to be cost-effective for industrial production.

The present study demonstrated the feasibility of an efficient method for extraction of PHB Alcaligenes eutrophus. Several non-toxic solvents (ethanol, propanol, dimethylsulfoxide, propylene carbonate, ethyl acetate and butyl acetate) were tested and it was found that ethylene carbonate is the most effective solvent for the PHB recovery. Testing was performed to examine the effects of temperature and heating incubation time on the selected solvent. The results showed temperature has more significant effect on biopolymer recovery than incubation time. Ethylene carbonate had a higher recovery level (97%) than standard chloroform extraction at 125°C and an incubation time of 25 min. At mentioned condition, PHB recorded the higher molecular weight of 1.2×106 compared to the chloroform standard procedure (1×106). The proposed procedure showed that ethylene carbonate is a non-halogenated good alternative to chlorinated solvents.

Keywords: Alcaligenes eutrophus, Ethylen carbonate, Recovery, Polyhydroxybutyrate.



[35]

SYNTHESIS AND ANTIDEPRESSANT EVALUATION OF COMPOUNDS CONTAINING 4-(1*H*-BENZO[d]IMIDAZOL-2-YL)-N-(SUBSTITUTED PHENYL)-4-OXOBUTANAMIDE

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ABSTRACT

A new synthetic strategy for the synthesis of novel 4-(1H-benzo[d]imidazol-2-yl)-N-(substituted phenyl)-4-oxobutanamide (3a-i) analogues is described and evaluated for their antidepressant activity. Reaction of 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoic acid (1) with 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoyl chloride (2) furnished novel 4-(1H-benzo[d]imidazol-2-yl)-N-(substituted phenyl)-4-oxobutanamide (3a-i). All the newly synthesized compounds were characterized by IR, 1H-NMR and Mass spectral analysis. The antidepressant activities of synthesized derivatives were compared with standard drug Clomipramine at a dose level of 20mg/kg. The compound 3a significantly reduced the duration of immobility time at 50mg/kg dose level considered to be highly promising when compared to the standard drug. Molecular docking studies revealed that the computational values obtained after docking calculation are in good agreement with the experimental values.

Keywords: Benzimidazole, Docking studies, Antidepressant activity, Clomipramine

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[36]

PRIMERS SUITABLE TO SCREEN FOR THE TYPE III SECRETION SYSTEMS IN BACTERIA

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ABSTRACT

Interaction of bacterial pathogens with host cells is particularly characterized by factors that are located on the bacterial cell surface or are secreted into the extra-cellular space. Among them, the Type Three Secretion System (TTSS) has been identified as the one that directly injects bacterial proteins to the cytosol of the eukaryotic cells. This mechanism has been identified in number of animals as well as plant pathogens, including highly virulent ones such as Yersinia pestis. The TTSS consists of a secretion apparatus, made up of ~ 25 proteins and an array of proteins released by this apparatus. The aim of this study was to synthesize suitable primer sets to screen for the presence of TTSS in bacteria. One of the highly conserved gene in the machinery, the type III ATPase gene was selected as the target gene to design the degenerate primers. Published sequences of Type III ATPase gene were aligned together using CLUSTAL W programme and four primers were designed (YN1, YN2, YN4, YN5) which would amplify a target sequence of 460-470 bp. Touch down PCR method was applied with different combinations of primers. On the identification of a target PCR product, it was cloned using the pCR 4-TOPO system (Invitrogen) and sequenced. Homology of the obtained DNA sequences were compared using Blastx programme. PCR reactions were optimised using two TTSS positive bacteria, Salmonella typhimurium and Yersinia pseudotuberculosis. Sequences confirmed the amplification of the type III ATPase in both positive controls rather than the closely associated flagella ATPase genes. Nevertheless, in the test organism Yersinia ruckeri, bacterial fish pathogen which has shown adhesion and invasive characteristics in the pathogenicity, TTSS was identified with a sequence homology of 80%, establishing suitability of the primers for screening for type III secretion systems. The least degenerate primer pair, YN1 (5'GARGGNCARMGNATGGGNATHTTYG 3') and YN4 (5'ARNACNGTRTARAANGCNGTDAT 3') was the most successful primer combination.

Keywords: primers, TTSS, bacteria, touch Down PCR

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[37]

AUXIN PRODUCTION AND BIOFERTILIZATION OF *Triticum aestivum* L. BY HALOTOLERANT RHIZOBACTERIA

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ABSTRACT

Salt-tolerant strains of Enterobacter asburiae, Bacillus thuringiensis, Moraxella pluranimalium and Pseudomonas stutzeri were evaluated for their ability to alleviate salt stress of wheat (*Triticum aestivum L.*) seedlings, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of P. stutzeri S-80 and B. thuringiensis S-26 was 190 and 183 nmolh-I, respectively. Maximum levels of auxin were recorded with P. stutzeri S-80 (107 µg ml-I) and E. asburiae S-24 (143 µg ml-I) under normal and salt-stressed conditions (0.25M NaCl), respectively with 500 µg ml-I L-tryptophan. Auxin response mediated by rhizobacteria was also demonstrated by microscopically assaying the transgenic auxin-responsive reporter DR5::GUS expression tomato (Solanum lycopersicum L. cv. MicroTom). In pot trials, seedlings fresh and dry biomass witnessed highly significant improvements of 1 and 2.2 folds, respectively, with M. pluranimalium S-29 (at 100 mM NaCl) and E. asburiae S-24 (150 mM NaCl), over control. At final harvest, maximum increase in number of tillers (up to 94%) and seed weight (up to 40%) were recorded with E. asburiae S-24 and M. pluranimalium S-29 at 200 mM salt stress. In conclusion, newly isolated strains of M. pluranimalium S-29, E. asburiae S-24 and P. stutzeri S-80 enhanced the growth of T. aestivum by mitigating the salt stress of plants.

Keywords: Halotolerant rhizobacteria, ACC-deaminase, bacterial auxin production, Salt stress alleviation, Plant growth promoting rhizobacteria



[38]

CONSTANT FLOW OF ELECTRICITY FROM JUMBO URINE

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ABSTRACT

In domestic as well as industrial energy demands, electricity has been the most abundant source of energy. Various ways has been implemented to generate, store and distribute electrical energy as and when needed. Continuous use of natural resources like fossil fuels in various ways has resulted in depletion of raw materials utilized for the generation of electricity. This has made man to look for more reliable and renewable sources for raw materials. Hence inventions and discoveries about solar power, and atomic power provided more energy sources.

Our need for sustainable and pollution free energy sources is rapidly growing and the demand for newer and better technology to generate energy is on the brim of boom. The current consumption rate of energy man exhibits shows a fast decrease in the energy sources and shows a rapid growth of demand for reliable renewable and natural energy sources. It is known to all of us that we can generate electricity from potato, and some research is already on going to generate electricity from cow's urine. My invention provides a much more resourceful source to generate electricity in the lowest possible budget, using Elephants urine as the electrolyte. The raw material can be abundantly obtained without much hassle from the southern regions of India as well as the most of the Asian countries. This procedure generates a byproduct, i.e., the decayed urine, which can be used as a bio fertilizer in such a way; this is not only a reliable source of energy but also a clean energy source.

Usage of renewable sources demanded a large area for installing the required apparatus to convert the energy into electricity, and hence is confined to industrial use alone. Solar power can be used to generate electricity on domestic basis, but in developing countries like India, it is very costly. This awareness made me think about a new and a much more simple way of energy conversion, using the basic science of electrolysis.



POSTER PRESENTATIONS





[01]

APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR EXOPOLYSACCHARIDE PRODUCTION FROM ENTEROCOCUS FAECIUM M 96.2

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ABSTRACT

The genus Enterococcus is a common LAB that often occurs in vegetables, dairy products and some other animal foods. These microorganisms are known to be able to resist pasteurization temperatures and adapt to different substrates and growth conditions. Enterococci have some biochemical properties such as lipolytic, proteolytic, and esterase activity, citrate utilization, production aromatic volatile compounds and exopolysaccharide. Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented dairy products such as yoghurt, drinking yoghurt, cheese, fermented cream, milk based desserts. Enterococci are considered probiotics, produce bacteriocins (enterocins), are active against listeria and other pathogens, and their use as costarter is recommended because they improve the flavor of fermented foods, mainly In this study, the synthesis of exopolysaccharide (EPS) from a probiotic Enterococcus faecium M96.2 were investigated. Exopolysaccharide (EPS) production by E. faecium was optimised using the response surface methodology (RSM). E. faecium strain was isolated from milk and identified by automatic riboprinter system. The isolate was identified as E.faecium M 96.2. The strain was grown in M17 medium at pH 6.5, 2% glucose, fructose, sucrose and lactose were added to the medium to study the influence of and mono disaccharides on the growth and production of exopolysaccharides (EPS). Maximum EPS production was 46.97 mg/L, and the optimised values of the three variables predicted for maximum EPS production included a temperature of 420C, lactose and yeast extract concentrations of %11.7 and %3.3 respectively.

Keywords: , exopolysaccharide, response surface methodology



[02]

A COMPARISON OF SOIL FUNGAL COMMUNITIES OF DRY ZONE AND WET ZONE FORESTS USING A METAGENOMIC APPROACH

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ABSTRACT

A preliminary soil metagenomic study was done to investigate and compare soil fungal diversity in two selected ecological zones in Sri Lanka. Wet evergreen forest, Kitulgala Makandawa forest reserve, was selected to represent wet zone and dry mixed ever green forest, Dimbulagala forest reserve was selected to represent the dry zone. Dimbulagal was specially selected because no research has been done in Dimbulagala for the past 30 years. Total of 9535 reads were obtained. After subjecting to quality assurance and filtering, data were analyzed in each community separately. Total of 815 Operational Taxonomic Units (OUT) were found in Kitulgala tropical rain forest and 1070 OTUs were found in Dimbulagala forest soil. Dimbulagala sample harbored high number of Operational Taxonomic Units (OTUs) compared to the Kitulgala sample. Members of Ascomycota were the highest in both samples and a high species and genus diversity was also observed. Many species have not been reported in Sri Lanka based on the previous literature. Unique species and genera were found in each sample inferring community level differentiation. This could be due to the differences in flora and fauna, climatic and geographic differences. As predicted, many fungal species were found with industrial or high economic value. Therefore, further research to isolate such species should be conducted to reap the benefits of these hidden treasures of our soils.



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[01]

POTENCY OF AGRICULTURAL WASTE FOR FORAGE IN TOBASA REGENCY

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ABSTRACT

Forage, which is provided by environment, is one of the important factors to consider in animal husbandry since the productivity of the livestock is 70% affected by environment rather than genetic factor whilst 60 up to 80% of cost production is allocated for producing and providing forage. In addition, the grazing land which simultaneously functions as the green resources for animal feed is getting lesser and lesser. In Tobasa regency, North Sumatera, Indonesia, the land used for farming is about 22,685 hectares which are used by the local people for growing paddy, cacao, corn, coffee, and other agricultural products. The waste produced as the result of these agricultural and farming activities can be used as animal feed or forage. This research aims to explore the efforts in reducing agricultural waste and utilize it as forage through fermentation process (ammonization and molasses) as the solution to the forage issues such as low nutrition and rough-fibred substance. The result of this research is expected to fulfill the needs of alternative animal feed and protecting the environment through diminishing agriculture waste.

Keywords: forage, agricultural waste, fermentation, livestock

