Pakistan Journal of Biochemistry and Molecular Biology

Preface

Fourteenth Biennial Conference of Pakistan Society for Biochemistry and Molecular Biology (PSBMB) was held during December 9-12, 2018 at Dr. A.Q. Khan Institute of Biotechnology & Genetic Engineering (KIBGE), University of Karachi, Karachi, Pakistan. Theme of the conference was "MOLECULAR BIOSCIENCES: RESEARCH AND INNOVATIONS". Hundreds of scientists, post-doctoral fellows and graduate students from all over Pakistan and other countries attended this conference.

Here we present abstracts of oral presentations delivered during the conference. Editorial board is grateful to the organizing committee of PSBMB 2018 for providing abstracts of oral presentations for publication in PJBMB.

Editorial board

Pakistan Journal of Biochemistry and Molecular Biology

Oral Presentations of 14th Biennial Conference of PSBMB (December 2018)

UTILIZATION OF RENEWABLE RESOURCES FOR BIOTECHNOLOGICAL APPROACH: PREPARATION OF CHITIN FROM CRUSTACEANS BIOWASTE

Aiman Pirzada, Tayyaba Asif, Bushra Zafar and Afsheen Aman
Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

Marine biome is constituted by materials with a wide range of unique characteristics and properties. The sustainable exploitation of renewable marine resources and the valorization of residues from marine origin, represents a highly fascinating platform for the development of novel biomaterials. Within the fishery industries, the waste management of crustacean biomass is a big issue causing massive environmental problem due to its lack of cost-effective utilization. There has been substantial interest in the development and commercialization of biodegradable products derived from marine biowaste. Crustacean shell constitutes of a polysaccharide 'chitin' which could be recovered from the crustacean exoskeletal structure. Chitin is an eco-friendly biopolymer with an extensive range of applications. The present study explains that how our natural marine resources could be utilized for the extraction of promising antimicrobial chitin-based polymers. In order to minimize marine biowaste, seafood processing waste samples will be collected from Pakistan Marine Fisheries to recover chitin and the purified chitin will be structurally characterized using different analytical techniques. To

determine the biotechnological potential of chitin, their antimicrobial activity will be analyzed using agar well diffusion assay and further by spectrophotometric measurements, which governs the vast array of applications for commercially valuable chitin-based products. The recycling of crustacean biowaste which is usually disposed of in landfills would be beneficial to the ecosystem as it can contribute to the bioeconomy as a valuable bioproducts.

ASSOCIATION OF *OMENTIN-1* GENE POLYMORPHISM WITH TYPE 2 DIABETES

Amber Khan, Sitwat Zehra, Ume Kulsoom, Syeda Nuzhat Nawab and Abid Azhar
Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering, University of Karachi, Karachi-75270, Pakistan

Type 2 Diabetes Mellitus (T2DM) is characterized by impaired glucose sensitivity resulting in hyperglycemia. According to World Health Organization, T2DM is 90% prevalent among all types of diabetes. The increasing burden of Type 2 diabetes has huge impact on global health. Omentin protein has anti-diabetic property hence; it is involved in managing blood glucose level. Omentin is produced by omental adipose tissues which are pathologically involved in the progression of T2DM. Objectives of this study are to identify genetic variations in *Omentin-1* gene and to evaluate the association between *Omentin-1* gene polymorphism with type 2 diabetes mellitus. Peripheral blood was collected from 150 type 2 diabetic patients and 100 healthy controls. Genomic DNA was isolated using conventional phenol chloroform

method. Specific segments of DNA were amplified by Polymerase Chain Reaction (PCR) and possible genetic polymorphism Val109Asp in *Omentin-1* gene was analyzed by Restriction Fragment Length Polymorphism (RFLP). The PCR- based RFLP results showed that *Omentin-1* gene polymorphism Val109Asp represents A to T transition. The wild type homozygous AA with variant heterozygous AT and homozygous AA genotypes were observed. The results will help to evaluate the unestablished frequency of genetic variants of *Omentin-1* gene in Type 2 Diabetic patients, which may help to identify critical genetic alterations as possible molecular targets for earlier diagnosis, improved prognosis and to develop better therapeutic protocols in future.

A STEP TOWARDS DATA COLLECTION FOR CEREBRAL PALSY AFFECTEES IN KARACHI: FINDINGS AND OBSTACLES

Ammara Rafique and Hajra Naz
Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan

Cerebral Palsy (CP) is a non-progressive neurodegenerative disorder, occurring in approximately 2 in 1,000 live born infants worldwide but paucity of data exists for our region. In our survey, almost 70 Karachi based health organizations were visited but only 658 cases were collected through a convenience sampling technique from 14 health organizations for the years 2010 to 2016. Of 658 Cerebral Palsy cases, 383 (58.20%) were male and 275 (41.79%) were female with 1.4:1 male to female ratio. Spastic Cerebral Palsy was found in 352 (53.49%)

followed by atonic, ataxic/mixed and athetoid/dyskinetic type of Cerebral Palsy accounting for 77 (11.70%), 22 (3.34%), and 67 (10.18%) respectively. 235 (35.71%) were found mildly affected, 112 (17.02%) were moderately affected, and 60 (9.11%) were severely affected. The comorbidities like epilepsy were found in 96 (14.58%), myotonic muscular dystrophy (MMD) found in 3 (0.45%), and Down's syndrome found in 2 (0.30%) along with CP. The Co-mitigating factors like ADHD found in 18 (2.73%), however, Autism was found in 9 (1.36%). The data collected for the 2010-2016, might serve to be an addition to the paucity of the data that exists for Cerebral Palsy patients in Karachi. In the present survey, a couple of rehabilitation centers, special schools, trusts, and hospitals either had high handed restrictions to access data at any level or they claimed that they were trying to establish data at the moment. Few had unjustified and questionable reasons for denying access to data. So, on the whole, our survey clearly enunciated that unfortunately there are inadequate systems to record data electronically and manually.

ANALYSIS OF THE ASSOCIATION OF *ADAM 12* GENE POLYMORPHISM WITH SUSCEPTIBILITY TO OSTEOARTHRITIS

Bushra Khan[1], Sehrish Fatima[1], Obaid Yusuf Khan[2] and Abid Azhar[1]
[1]Dr. A. Q. Khan Institute of Biotechnology & Genetic Engineering (KIBGE),
University of Karachi, Karachi
[2]Department of Genetics, University of Karachi, Karachi, Pakistan.

Osteoarthritis (OA) is considered as the most common joint disorder, causing pain and disability in affected individuals, with very high incidence and prevalence across the globe. Several risk factors have been involved in OA development and progression including age, gender, obesity, injury, family history and genetic variations. OA has a substantial hereditary component and the associated genes tend to be related to the process of synovial joint development. Several studies suggest that *ADAM 12* gene polymorphism *rs1044122* representing the synonymous polymorphism (*Ala825Ala)* is primarily associated with OA in different ethnic groups. Therefore, this study was designed to investigate the association of *ADAM 12* gene polymorphism *rs1044122* with susceptibility to OA. To execute the case-control study, detailed history with clinical and radiographic examinations were recorded in patients' data collection form. Blood samples were collected with equal number of patients (n=400) and controls (n=400). DNA was extracted by standard phenol-chloroform method. Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for genotypic analyses. Genotype and allele frequencies were compared using odds ratio and chi-square statistics. It was observed that the SNP

rs1044122 showed significant association with OA in local population. The genotype distribution of OA patients differed significantly from that of controls ($\chi 2$=31.36, p<0.001). In addition, C allele was found more frequent in OA patients as compared to controls and the difference was also statistically significant ($\chi 2$=29.97, p<0.001). These data suggest that *ADAM 12 gene polymorphism rs1044122* might be associated with OA susceptibility among local population. However, further investigation is required to confirm the observations.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF INVERTASE ISOZYMES REGULATING SUCROSE MECHANISM IN HEAT SHOCK SUGARCANE

Faisal Mehdi[1], Nabeela Afzal[1], Abid Azhar[2] and Saddia Galani[2]
[1]Agriculture and Agribusiness Management University of Karachi, Karachi-75270, Pakistan
[2]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering University of Karachi, Karachi-75270, Pakistan

Impact of high temperature on biochemical changes such as sugar profiling with respect to invertase isozymes were evaluated in two cultivars S2003-US-633 and SPF-238 under thermal stress. For this purpose, sugarcane tissues (formative, grand growth and maturity) were collected from these two cultivars at different temperature regimes i.e. control, heat shock treatment (45 ±2 °C) and recovery for different time episodes i.e. T24 hrs, T48 hrs and T72 hrs. Total sugars, reducing sugars, non-reducing sugars and activities of invertase isozymes (cell wall, vacuolar and cytoplasmic) were quantified. The qualitative analysis of isozymes was done through Native-PAGE and differential staining. Results demonstrated that cultivar S2003-US-633 under heat stress displayed reduced cell wall and cytoplasmic invertase activities along with reducing sugars at grand growth and maturity stages. While enhanced total and non-reducing sugar contents were evident depicting the upregulation of other sugar metabolizing enzymes. Zymography analysis revealed multiple bands of cytoplasmic and vacuolar invertase isoforms at grand growth and maturity stages while cell wall invertase isoforms were evident at vegetative stage in S2003-US-633 cultivar. It can be concluded that invertase isoforms played

key role in sucrose metabolism in sugarcane stalk and can be used as a useful marker for screening high sugar yielding sugarcane varieties.

GENETIC VARIANT OF *CASP8* D302H: RELATIONSHIP WITH BREAST CANCER OUTCOMES AND DEVELOPMENT

Faria Fatima[1], Abdul Hameed[2], Ghulam Haider[3], Abid Azhar[1] and Saima Saleem[1]

[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

[2]Institute of Biomedical and Genetic Engineering (IB&GE), Islamabad, Pakistan

[3]Department of Oncology, Jinnah Post-graduate Medical Center, Karachi, Pakistan

Check points of apoptosis played integral part in cellular growth. Genetic changes which inactivate these check points may result in tumorigenesis. Caspase-8 is a cysteine protease and mediates apoptosis through death receptors. Cellular death pathway, one of the crucial defense systems against malignant transformation and progression. Polymorphism in *Casp8* gene (rs1045485) leads to the substitution of aspartate (D) to histidine (H) at 302 amino acid, may result in auto processing and interaction with antiapoptotic molecules which cause carcinogenesis. Current study aims to investigate the role of *Casp8* D302H polymorphism in breast cancer development and progression. After the informed consent 100 tissue samples were taken from diagnosed breast cancer cases. Genotyping of D302H of *Casp8* was done by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) via

Hin1II and confirmed by direct DNA sequencing. Obtained data was analyzed by statistical and bioinformatics tools. The polymorphism *Casp8* (rs1045485) results in substitution of D to H forms two alleles i.e. G allele (major allele) and C allele (minor allele). Tissues samples predominantly showed GG genotype. However, GC genotype is represented by only 1% of cases whereas CC genotype is not present. Caspase 8 has distinctive feature as an initiator of death receptor in apoptotic responses. It has been suggested *Casp8* (D302H) CC and CG variants might reduce the odds of breast cancer development. Low frequency of C allele in breast cancer tissue samples signifies its emergence, as a somatic cell mutation rather than germline. Moreover, increased number of samples with further investigative approaches are required to achieve more insights about the molecular mechanisms of *Casp8* involved in breast cancer outcomes and progression.

BACTERIOCIN FROM *LACTOBACILLUS PLANTARUM*: PRODUCTION, PURIFICATION AND *IN VITRO* APPLICATIONS

Fariha Ibrahim, Syeda Bushra Zafar, Afsheen Aman and Asma Ansari
Dr A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

In the past few years, pathogenic bacteria have acquired antibiotic resistance against multiple drugs. The problem is not only due to the microbes that developed different ways to resist effective antibiotics, but also it is because of the over prescribing and inadequate use of the drugs. This problem can be

resolved by the discovery of natural antimicrobial compounds having broad spectrum of inhibition against multidrug resistant organisms. One of the alternatives to this issue is bacteriocin. Utilization of bacteriocins produced by lactic acid bacteria (LAB) has received great attention. Bacteriocins can not only be used as an alternative therapeutic agent but also as a probiotic. In the present study, bacteriocin producing *Lactobacillus plantarum* KIBGE-IB45 was identified through 16S rDNA sequence analysis. Current study is an effort for the enhanced production of bacteriocin using combinatorial strategy with the plausible *in-vitro* applications. To obtain maximum bacteriocin yield, different production parameters were optimized and maximum bacteriocin production was achieved at 30˚C with initial pH-8.0 of MRS medium after 20 hours of incubation. Moreover, localization of Bac+ gene was determined using plasmid curing technique. It was found that the gene responsible for the bacteriocin production was located on chromosome. Afterwards, bacteriocin was purified and its cytotoxic analysis was determined. Mode of action and antibacterial spectrum of bacteriocin revealed that this bacteriocin has a potential to control and inhibit multidrug resistant pathogens. Therefore, it can be used as an alternative therapeutic agent in pharmaceutical industries.

ANTIPROLIFERATIVE POTENTIAL OF QUINAZOLINE DERIVATIVE ON INTRACELLULAR SIGNALING CASCADE IN ORAL SQUAMOUS CELL CARCINOMA

Fariha Tanveer, Amber Ilyas and Shamshad Zarina
National Centre for Proteomics, University of Karachi, Karachi, Pakistan

Cancer is the second leading cause of death around the world. Carcinomas of epithelial lining tend to pose serious treatment challenges in clinical practice. Oral Squamous Cell Carcinoma (OSCC) stands eighth in context of mortalities. About 500,000 new cases are diagnosed annually around the globe with higher incidence rate in South-East Asia. Epithelial carcinomas are often correlated with perturbation of growth factors and receptor mediated signaling. Over expression of Epidermal Growth Factor and Receptor (EGF and EGFR) signaling is the most frequently observed molecular event in carcinomas of squamous epithelial origin. More than 90% of OSCC cases are reported with up-regulation of EGF and EGFR. Therefore, it is suggested that inhibitors of these pathways are likely to act as potential therapeutic agents. The current study was aimed to determine the role of Quinazoline derived compounds in intracellular signaling cascade. To check cytotoxic potential, we incubated OSCC cell line with different concentrations of Varlitinib for 24, 48 and 72 hours. We observed 50 % inhibition of cell viability in 24 hours. We also checked apoptotic activity with DNA demethylation agent, 5AzaC and DNA Alkylating agent Temozolomide. In addition to this, Two-Dimensional Gel Electrophoresis (2D-GE) was performed to determine expression pattern of proteins in control and treated groups

of cells. Furthermore, we checked Real Time gene expression pattern of SOCS1, Akt1, MAPK, p53, p21, in treated and control groups. Our results indicated inhibition of metastasis and induction of apoptosis, suggesting therapeutic benefits of tested compounds.

ATTENUATION OF MIDAZOLAM-INDUCED CONDITIONED PLACE PREFERENCE IN RATS BY BUSPIRONE

Huma Ikram[1], Sarah Tasneem[2], Darakhshan J. Haleem[1,2]
[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi-75270, Pakistan.
[2]Neuroscience Research Laboratory, Dr Panjwani Center for Molecular Medicine and Drug Research-ICCBS, University of Karachi, Karachi-75270, Pakistan

Although there is a well-established role of dopamine in the addictive and reinforcing effects of drugs of abuse, various studies have also reported involvement of serotonin (5-hydroxytryptamine; 5-HT) in the reinforcing and addictive effects of drugs of abuse. Midazolam is a familiar benzodiazepine (BDZ) commonly used in the emergency department to provide sedation prior to procedures such as laceration repair and reduction of dislocations. However, despite its vast clinical/ therapeutic implications, midazolam produces addictive effects upon repeated administration. The present study is designed to determine the role of serotonin-1A receptors in the reinforcing effects of midazolam. Rats received one (daily; 2.5 mg/kg) injection of midazolam on alternate days (for 12 days) and midazolam was paired with placement in one compartment

of conditioned place preference (CPP) apparatus. Buspirone (1.0 mg/kg) was injected daily and was not paired with any of the two compartments of the CPP apparatus. We tested the hypothesis that desensitization of somatodendritic 5-HT1A receptors upon repeated administration of buspirone, may attenuate midazolam-induced addictive effects. Administration of midazolam at a dose of 2.5 mg/kg increased number of entries as well as time spent in the drug-paired compartment of the CPP apparatus. Coadministration of buspirone at a dose of 1.0 mg/kg attenuated midazolam-induced reinforcement. Buspirone at the dose of 1.0 mg/kg did not alter the activity of saline injected animals. Results suggest that buspirone may attenuate midazolam-induced addiction. Findings may help in increasing the therapeutic potential of BDZs with minimal/ reduced side effects.

MUTATIONAL ANALYSIS OF HOTSPOT REGION OF EXON 11, *BRCA1* GENE IN BREAST CARCINOMA AMONG THE PATIENTS OF BALOCHISTAN, PAKISTAN

Kiran Rani[1], Naheed Sajjad[1], Neelam Hashmi[1], Asma Yousafzai[2] and Nisar Ahmed[2]

[1] Sardar Bahadur Khan Women's University, Quetta, Pakistan

[2] Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan

Breast cancer is the global burden occurring most commonly and suggested to be the second foremost cause of death among women worldwide. About one million new breast cancer cases each year, 55% death cases and 45% diagnosed cases are reported to be from middle and low income countries. Aim in this study was to investigate gene

BRCA1 exon 11 mutations related to breast cancer from Balochistani population. Almost 50 breast cancer patients from different ethnic groups of Balochistan and 50 normal individuals were enrolled from tertiary care hospitals. Mutational analysis in gene BRCA1 exon 11 related with breast cancer,was performed. Sequence analysis revealed one pathogenic frame shift (c.2980InsA, p.Cys994) variants which was novel, one reported pathogenic frame shift mutation (c.1962InsA, p.Tyr655), two reported heterozygous missense mutations (c.1067A>G, p.Gln356Arg; c.2042G>A, p.Ser681Asn) both pathogenic and non-pathogenic were also identified in current study. It was suggested that these variations result the synthesis of a premature protein with lack of function by affecting the domains of resulting protein.

DNA METHYLATION OF *MST1*: AN EPIGENETIC ANALYSIS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Madiha Kanwal[1], Abdul Hameed[2], Ghulam Haider[3], Abid Azhar[1] and Saima Saleem[1]

[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

[2]Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan

[3]Jinnah Postgraduate Medical Centre (JPMC), Karachi, Pakistan

Head and neck squamous cell carcinoma (HNSCC) is one of the cancers that are strongly associated with tobacco consumption and its carcinogenic components. These components are known to elicit genetic and epigenetic alterations in numerous cancers. Hippo cell signaling

pathway regulates cell proliferation, differentiation, growth, and death. Due to DNA methylation of *MST1* which is a tumor suppressor gene of hippo pathway, transcription factor cannot bind to promoter region and gene is not transcribed due to which lower expression or gene silencing of *MST1* occurs, which may lead to cancer formation. Use of tobacco might be one of the leading causes of DNA methylation that affects hippo pathway in HNSCC development. The aim of study is to analyze DNA methylation of *MST1* gene of hippo pathway and its association with HNSCC. Total 156 patients of HNSCC were included in this study and divided into two groups, tobacco addicted group of patients and non-addicted group of patients. Demographic data analyses were performed between the two groups of patients. DNA methylation of *MST1* was investigated by DNA extraction and bisulfite modification, methylation-specific PCR and agarose gel electrophoresis. The findings revealed that HNSCC in addicted and non-addicted patients varies in a number of different aspects. Particularly patient gender, age, income and site of occurrence. DNA hypermethylation was found in the two groups of patients. This study may increase our understanding of epigenetics of HNSCC. By giving an insight into the processes of HNSCC development, the understanding of epigenetic information might be translated into clinical benefit in HNSCC as biomarkers.

PLATELET AGGREGATION INHIBITORY ACTIVITY OF ANTHRACEN-1,2-10-TRI-O-ACETATE: NEWLY SYNTHESIZED DERIVATIVE OF ANTHRAROBIN

Mehreen Lateef[1], Abid Azhar[2], Huzoor Akbar[3], Bina S. Sidduqui[4]

[1]Bahria University Medical and Dental College, Bahria University, Karachi, Pakistan

[2]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

[3]Department of Biomedical Sciences, Heritage College of Osteopathic Medicine, Ohio University

[4]HEJ Research Institute of Chemistry, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi-75270, Pakistan

The inhibition of platelet aggregation by synthetic and naturally isolated compounds has gained attention due to increasing demand of pharmaceutical industry regarding concerns of antiplatelet drug with no or less side effect. In search to explore more safe and potent antiplatelet compounds, this study is planned to explore antiplatelet activity of anthrarobin derivatives. Two new derivatives of Anthracen-1,2-10-tri-acetate (anthrarobin) were synthesized and pharmacologically evaluated for platelet aggregation and analgesic activities. Anthrarobin was acetylated with acetic anhydride in presence of pyridine and 1, 10 dihydoxyanthracen-2-acetate (1) and Anthracen-1, 2-10- tri-acetate (2) were obtained as one of the two derivatives. These acetylated derivatives of anthrarobin, (1) and (2) were analyzed for *invitro* antiplatelet activity in washed human platelets and platelet rich plasma. It is found that Anthracene-1,2-10-triacetate (2) significantly inhibited platelet aggregation at the concentration of 10µM induced by threshold aggregating concentration of thrombin (0.03U), collagen (1.2 µg/ml), ADP (1.75µM), U46619 (0.8µM) and

PMA (6.2ηM). Beside, 1, 10 dihydoxyanthracen-2-acetate (1) showed no or very little inhibition of aggregation induced by above mentioned inducers. This antiplatelet compound (2) was further tested against analgesic and antinflammatory activity at selected doses (1, 3 and 10 mg/kg of body weight in mice) and found to have potent analgesic and antinflammatory properties. These preliminary findings suggest anthracen-1, 2-10-tri- acetate (2) possesses pharmacological activity and could be potential templates for the development of new anti-platelet agents.

INTEGRATED CHEMINFORMATICS - MOLECULAR DOCKING APPROACH TO DRUG DISCOVERY AGAINST HCV

Muhammad Faraz Anwar[1,2], Ramsha Khalid[3], Alina Hasanain[4], Sadaf Naeem[3], Shamshad Zarina[1], Syed Hani Abidi[2] and Syed Ali[5]
[1] National Center for Proteomics, University of Karachi, Karachi-75270, Pakistan
[2] Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan
[3] Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan
[4] Medical College, Aga Khan University, Karachi, Pakistan
[5] Department of Biomedical Sciences, Nazarbayev University School of Medicine, Nazarbayev University, Astana, Kazakhstan

In the current study, we present an integrated *in silico* cheminformatics-molecular docking approach to screen potential therapeutic compounds against HCV. Fluoroquinolones have been shown to inhibit HCV replication by targeting HCV NS3-helicase. Based on this observation, we hypothesized that natural analogs of

fluoroquinolones will have similar or superior inhibitory potential, while having potentially fewer adverse effects. To screen for natural analogs of fluoroquinolones, we devised an integrated *in silico* Cheminformatics-Molecular Docking approach. We used 17 fluoroquinolones as bait reference, to screen large databases of natural analogs. 10399 natural compounds and their derivatives were retrieved from the databases. From these compounds, molecules bearing physicochemical similarities with fluoroquinolones were analyzed using a cheminformatics-docking approach. Twenty compounds were picked up using our cheminformatics approach. Molecular docking analysis showed 32 amino acids in the HCV NS3 active site that were most frequently targeted by fluoroquinolones and their natural analogues, indicating a functional similarity between the two groups of compounds. This study describes a speedy and inexpensive approach to complement drug discovery. The *in silico* analyses we used here can be employed to short-list promising compounds/putative drugs that can be further tested in wet-lab.

MURINE MODEL OF BRONCHIAL ALLERGY AND ALLERGIC RHINITIS TRIGGERED BY THE AIRBORNE POLLEN AND SPORES

Muneeba Khan[1], Anjum Perveen[1], Saddia Galani[2], Sana Aijaz[3]
[1]Centre for Plant Conservation, University of Karachi, Karachi-75270, Pakistan
[2]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan
[3]Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

Airborne allergens cause the inflammation of inner linings of windpipes that carries air by nose to the lungs. This swelling of windpipes in known as allergic bronchitis. Airway irritants are major cause of allergy and asthma in hypersensitive individuals. The present research work is designed to develop a murine animal model for the testing of selected allergens. Pollen of *Typha* sp. and spore of *Aspergillus* sp. were used for intranasal sensitization of BALB/c mice. Allergy symptoms were recorded. Conformation of allergy symptoms was done by WBC count (differential). Data was analysed by using Systat Software, Inc. SigmaPlot for Windows ver. 13.0. Pollen protein analysis was also done by SDS-PAGE analysis. Our study revealed that intranasal sensitization by pollen grains and fungal spores caused allergy in mice indicated by puffiness of eyes; sneezing; itching; and decrease in physical activity. Blood test also showed elevated level of eosinophils in sensitized mice (p-value > 0.01). SDS-

PAGE analysis of *Typha* pollen also showed the presence of low molecular weight proteins (20 KDa- 55 KDa). The presented data strongly suggest that the *Typha domingensis* and *Aspergillus niger* are possible aeroallergens. This study would also aid in allergy suffering patient therapy.

APPLICATION OF PLANT EXTRACTS (NEEM AND EUCALYPTUS) REGULATING OXIDATIVE DEFENSE SYSTEM IN TOMATO SEEDLINGS INOCULATED BY *RHIZOCTONIA SOLANI*

Nabeela Afzal[1], Faisal Mehdi[1], Saleem Shahzad[1] and Saddia Galani[2]

[1] Department of Agriculture and Agribusiness Management, University of Karachi, Karachi-75270, Pakistan

[2]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

Replacement of synthetic fungicides with plant extracts is an effective strategy in disease management. The current study aimed to investigate the ameliorative effect of aqueous extracts of neem and eucalyptus applied on 15 days old diseased tomato seedlings of two cultivars (Rio Grande & Rio Fuego). After 45 days the plants were harvested to assess the oxidative damages, antioxidant enzymes and proteins expressions. Result revealed that disease stress significantly increased the contents of lipid peroxidation (MDA), hydrogen peroxide (H_2O_2) and activities of superoxide dismutase (SOD), peroxidase (POD) enzymes in plants of both varieties as compared to control. Application of neem and eucalyptus extracts showed significantly decline in MDA, H_2O_2 content by

hyperactivity of SOD and POD in Rio Grande as compared to other. Protein expression indicated that pattern of low molecular proteins were evident in diseased and plant extracts treated sample as compared to control. These finding suggesting that the plant extracts application as novel strategy involved in inducement of systemic acquired resistance by the expression of defense related proteins and antioxidant defensive enzymes.

ANATOMICAL AND MOLECULAR ABERRATIONS (*GATA 4* GENE) IN VENTRICULAR SEPTAL DEFECT (VSD) PATIENTS IN KARACHI, PAKISTAN

Nazia Shahab[1], Hussain Ahmed[2], Afsheen Arif[2], Khalida Parveen[1] and Najma Patel[3]
[1]Department of Anatomy, Dow International Medical College, Dow University of Health Sciences, DUHS, Karachi, Pakistan
[2]Dr. A. Q. Khan Institute of Biotechnology & Genetic Engineering (KIBGE), University of Karachi-75270, Pakistan
[3]National Institute of Cardiovascular Diseases, (NICVD), Karachi Pakistan

Congenital Heart Disease (CHD) is among the most common anatomical disorders manifested at birth, among them ventricular septal defects (VSD) ranked among the top in terms of frequency. The present study investigated the degree of anatomical anomalies of heart in children of 0 to 10 years in non-syndromic VSD. Sonographic examinations conducted in all the patients examined to quantify several ventricular parameters such as size and site of septal defects, dimensions of ventricles, pulmonary artery hypertension, right coronary cusp prolapse etc. Subsequently the values were plotted in the graphs to delineate the pattern of deviations in different

anatomical characteristics of heart during VSD. The data was further exploited to increase the diagnostic accuracy of VSD and/or its severity. Additionally, as many other fields of life sciences, the study of anatomy is also in the phase of transition, where molecular and genetic studies are often considered a worthy component of research in anatomy. Another facet of the study was the genetic and/or molecular investigation of the VSD. *GATA4* sequential variants are explored in fifty patients. Molecular structural models of GATA4 wild type and mutant proteins constructed to unravel the structural changes between the wild type and mutant proteins. The functional impact on the mutations that underpins the incidence of VSD were further explored by molecular networking and molecular interaction analysis.

In total, the study provides the data regarding the anatomical and molecular aberrations in connection to VSD but also provided the baseline information to design future investigations and diagnosis for better accuracy and resolution.

RNA ISOLATION EFFICACY: COMPARISON OF DIFFERENT RNA EXTRACTION METHODS FOR *CITRUS TRISTEZA* VIRUS (CTV) DETECTION

Nesheman Huma[1], Shagufta Sahar[2], Arif Ali Chishti[1], Abid Azhar[1] and Saddia Galani[1]

[1]Dr. A. Q. Khan. Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

[2]Department of Agriculture and Agribusiness Management, University of Karachi, Karachi-75270, Pakistan

Efficient RNA recovery methods with high yield and quality are required for the RNA based viral detections. But due to high phenolic compounds, difficulties in extracting RNA from woody plants has remained an important problem while performing molecular studies. The polysaccharides and polyphenols usually co precipitate along with the nucleic acids which makes the RNA quality compromised for further downstream applications. In this study, we have optimized four different extraction methods based on TRIzol, SDS/phenol, CTAB and a commercial kit for efficient RNA recovery from citrus species. These methods were previously reported but for limited Citrus specie and were only effective against the samples present in fresh condition. However, in this experiment different citrus specie and samples at stored conditions are also well studied. The evaluation of RNA isolation methods includes the quality, concentration and RNA yield of these samples. The results indicated that among these four methods the quality of SDS/Phenol, CTAB and kit-based method indicate a A260/A280 ratio around 2.0-2.1 in both fresh and stored samples while concentration varied among

different methods. Substantially, visualization of pure and nondegraded RNA was also performed in which intact bands in the fresh samples as compared to the stored ones were present. To further validate the specificity and efficacy of the extracted RNA samples one step and two step PCR results were also evaluated along with internal controls specific for messenger RNA (mRNA). Consequently, RNA recovery methods influence the resultant RNA quality and quantity which crucially effect downstream molecular biology experiments.

SYNERGESTIC EFFECT OF GOAT MILK AND *THUJA OCCIDENTALIS* IN TREATMENT OF CYSTITIS CAUSED BY *CANDIDA ALBICANS*

Owais Fareed, Drousham Baloch and Saima Tauseef
Federal Urdu Univesity of Arts, Science and Technology, Karachi, Pakistan

Candidiasis are life threatening systemic fungal diseases especially of GIT, mucous membrane lining various body cavities and genital track. Due to increasing resistance of candida to existing drugs, it is very important to look for new strategies helping treatment of such fungal disease. Candida Albicans mostly attack on immunocompromised host and infected HIV person and the person who is on chemo therapy and having immunosuppressant. C. Albican has a property to invade the host and evade from host adaptive immune response.

In vitro we study on Hyphae formation assay and adhesion assay. In vivo we study on mice and in man. Patient suffering from cadidiasis and yeast growth in urine culture,

give orally goat milk and Thuja occidentalis consecutive three days, test perform before and after therapy. In mice we noticed that TLC increasing after giving Thuja Occidentalis orally. In antibody production the retentate fraction produced a concentration depended increase in the number of antibody producing lymphocyte in the hemolytic plaque assay in vitro.
Hyphae formation assay on capric acid inactive fraction of 2 to 5 percent yeast cell. Hence capric acid is responsible for inhibition of C. Albican's filamentation and biofilm formation. In man after therapy no growth in urine culture and clear ultra sonography. In mice Thuja increases TLC.
We noticed that the synergism of Goat Milk and Thuja occidentalis have effect on filamentous growth of candida and inhabit the adhesion and biofilm formation and stimulating the immunity to take into phagocytosis process.

STATISTICALLY ENHANCED PRODUCTION AND CHARACTERIZATION OF EXOPOLYSACCHARIDE FROM INDIGENOUSLY ISOLATED BACTERIAL SPECIES

Rabeeya Iftikhar, Zahida Shabbir, Nadir Naveed Siddiqui and Afsheen Aman
Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

Biotechnological applications of microbial exopolysaccharides (EPS) have been the major inspiration towards exploration of new microbial communities that can produce biopolymers. EPS are produced by variety of bacterial, algal and fungal species. Among them bacterial EPS have broad range of applications in food, pharmaceutical, cosmetics and petrochemical industries.

Advancement in microbial exploration techniques have assisted in discovering new microbial species which can produce valuable and novel exopolysaccharides. Most commonly known bacterial species that synthesize EPS belongs to genus *Streptococcus* (Mutans), *Leuconostoc* (Dextran), *Xanthomonas* (Xanthan), *Weissella* (Dextran), *Oenococcus* (Dextran), *Acinetobacter* (Cellulose) and *Azotobacter* (Alginate) species. Considering this fact in view, the current research is designed for the exploration of EPS producing microbes using indigenously isolated lactic acid bacterial species. Multivariate statistical model assembly will be designed for the optimized production of EPS using Design of Experiment (DoE). DoE is a preferred technique over conventional one-factor-at-a-time approach because DoE can purposefully consider several fermentation parameters concurrently in less experimental runs. The current study will determine the optimal level of interactions between various fermentation variables for EPS. The EPS produced will be further investigated for its physicochemical properties. Detailed structural characteristics in terms of composition and linkage will also be analyzed to study its innovative applicability in different fields. The newly identified EPS will be tested against mammalian cell lines in order to study its cytotoxicity for its potential commercial applications.

ROLE OF *SOD1* 50-BP INSERTION/DELETION POLYMORPHISM OF PROMOTOR REGION IN THE SUSCEPTIBILITY OF TYPE 2 DIABETES MELLITUS

Sanober Kafeel[1], Syeda Nuzhat Nawab[1], Zehra Manzoor[3], Ashar Fawad[2], Sitwat Zehra[1] and Abid Azhar[1]

[1] Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karfachi-75270, Pakistan

[2] Baqai Institute of Diabetes and Endocrinology (BIDE), Karachi, Pakistan

[3] National Center for Proteomics (NCP), University of Karachi, Karfachi-75270, Pakistan

In hyperglycemia, free superoxide radicals ultimately alter the structure of biomolecules under oxidative stress in type 2 diabetes mellitus (T2DM). Thus, it leads to reduce the scavenging activity of antioxidant enzyme SOD1 which normally functions to detoriate superoxide radicals into less noxious form H_2O_2 and H_2O. Therefore, this study aims to explore the relationship of *SOD1* 50bp Ins/Del polymorphism with the susceptibility of T2DM. Approximately 178 diabetic and 180 control blood samples were recruited from Baqai Institute of Diabetes and Endocrinology. Baseline characteristics and clinical profile was also retrieved from each subject. DNA was extracted for genotypic analysis of *SOD1* 50bp Ins/Del polymorphism performed on optimize conditions of polymerase chain reaction (PCR). Interpretation of the data was accomplished by statistical analysis using software SPSS version 20.0. Allelic and genotypic distribution was not found to be statistically significant in *SOD1* 50bp Ins/Del polymorphism among the groups (p=0.32). While mutant D allele possess a prompting role in the progression of disease and I allele

provides a shielding effect. Although genotypic frequency of ID was found to be higher in diabetic subjects than controls (22% vs. 17%). Findings suggest that *SOD1* 50-bp Ins/Del polymorphism is not significantly associated with the susceptibility of T2DM. However, further genetic studies on population scales are needed to elucidate the detailed role of this polymorphism as a risk factor for T2DM.

GENETIC VARIATIONS OF *P21* GENE: RELATIONSHIP WITH ORAL SUBMUCOUS FIBROSIS (OSF) AND ORAL SQUAMOUS CELL CARCINOMA (OSCC) IN PAKISTAN

Syed Aqib Ali Zaidi[1], Saima Saleem[1], Zubair Ahmed Abbasi[2], Uzma Zareef[3], Nadia Chughtai[4], Jehan Alam[5] and Abid Azhar[1]
[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi.
[2]Jinnah Sindh Medical University, Karachi, Pakistan
[3]Liaquat College of Medical and Dentistry, Karachi, Pakistan
[4]Karachi Medical and Dental College, Karachi, Pakistan
[5]Jinnah Postgraduate Medical Centre (JPMC), Karachi, Pakistan

Oral submucous fibrosis (OSF), a pre-malignant disorder that is characterized by the excessive deposition of collagen with inflammation of epithelia, is the most prevalent precancerous condition in Pakistan. It has been found that genetic variations in tumor suppressor genes may involve in the transformation of OSF into OSCC. *p21* is a member of tumor suppressor gene family and involved in the regulation of *TP53* in cell cycle arrest. Genetic variations in *p21* may exerts some changes in OSF that shapes OSCC. Current study is focused to find out the genetic association between OSF and OSCC by screening and comparing the reported genetic variations in *p21* gene. After informed consent, a

total number of 50 samples were collected from OSF and OSCC patients each, from the local hospitals of Karachi. The patients were selected on the basis of their chewing habit history of areca nuts, betel nuts, betel quid, gutka and/or manpuri. The DNA was extracted by standard phenol chloroform method. Targeted mutations were detected through tetra primers amplification refractory mutation system (T-ARMS) PCR and examine on agarose gel electrophoresis. In this study, cytosine (C) is a wild allele substitutes by adenine (A) allele and changes serine (ser) to arginine (arg). In OSF and OSCC samples these alleles showed three genotypes: (A/A) homozygous, (A/C) heterozygous and (C/C) homozygous. These variations of *p21* gene in OSF may act as a biomarker that would be helpful in the diagnosis of OSCC and might improve the understanding of development and progression of OSCC.

MULTIVARIATE APPROACH: BIOPROSPECTING AND STATISTICAL OPTIMIZATION OF PRODUCTION PARAMETERS FOR A NEWLY ISOLATED DEXTRANSUCRASE PRODUCING *WEISSELLA CONFUSA* KIBGE-IB38

Syeda Bushra Zafar, Tayyaba Asif, Fariha Ibrahim and Afsheen Aman
Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE),
University of Karachi, Karachi-75270, Pakistan

Dextransucrase is responsible for the production of a commercial polymer, dextran, Dextran and its derivatives have potential in biotechnological sectors. Therefore, there

is a great interest in exploring the biodiversity and developing new strategies for the cost-effective biosynthesis of this valuable enzyme. In the current study, various indigenous sources were explored to discover new dextransucrase producing strain, followed by statistical optimization of the production parameters for upregulation of its biochemical processes. Firstly, some new lactic acid bacteria of genus *Leuconostoc, Weissella* and *Streptococcus* were isolated and screened for dextransucrase. The unique properties of dextran produced from *Weissella* make this strain a preferred candidate for dextransucrase biosynthesis. The fermentation parameters of the selected isolate *Weissella confusa* KIBGE-IB38 was optimized using a multivariate statistical method. Two mathematical models, Plackett-Burman and Response Surface Methodology were constructed using Design Expert® software. Plackett-Burman was initially performed to screen nine fermentation parameters including time, temperature, pH and different concentrations of sucrose, yeast extract, peptone, dipotassium hydrogen phosphate, sodium chloride and calcium chloride. Among them, pH, time, sucrose and peptone exhibited significant positive impact on enzyme yield thus, selected for further optimization of the levels of these parameters for enhanced dextransucrase yield. The overall results revealed that after implementation of these models, enzyme titer enhanced up to 4.39-fold (12.0 DSU ml^{-1} to 52.75 DSU ml^{-1}) under optimized conditions.

EXPLORING ANTIFUNGAL POTENTIAL OF CHITINASE UTILIZED AS BIOPESTICIDE

Tayyaba Asif, Aiman Pirzada, Fehmina Khan, Asma Ansari and Afsheen Aman
Dr. A. Q. Khan Institute of Biotechnology & Genetic Engineering, (KIBGE), University of Karachi, Karachi-75270, Pakistan

Crop cultivation is vital to fulfill nutritional requirements of human beings but the agricultural sector is always at a high risk of being attacked by phytopathogens. Fungal plant diseases are one of the major concerns of agricultural production. Soil borne pathogenic fungi attack most of the economically important crop plants. Mycolytic enzymes (chitinases, proteases and glucanase) producing microorganisms may help in solving these problems. Chitinases can enhance the plant's defense system as they act on chitin, a major component of the cell wall of pathogenic fungi, and reduce the fungi inactive without any negative impact on the plants. Along with strengthening plant defense mechanisms, chitinases also improve plant growth and yield. These microorganisms have ability to lyse the fungal cell wall and also have the potential to manage the chitinous waste by producing chitinases. Many chitinolytic microorganisms have potential to control fungal plant pathogens. The present study identified chitin as a reliable substrate for chitinase production by *Glutamicibacter uratoxydans*. Chitinolytic bacteria were isolated from indigenous sources using crustacean waste biomass. Colloidal chitin was incorporated as a sole carbon source for the production of chitinase. The bacterial species showing clear zone of hydrolysis in colloidal chitin agar

medium were selected for further identification. Taxonomic studies and 16S rDNA sequence analysis was performed, and production parameters were optimized. The antifungal potential of the chitinase produced by *G. uratoxydans* was performed to utilize the chitinase as an effective biopesticide. Chitinase is a promising tool against phytopathogens, the current demand is to enhance its production to increase its applicability.

VITAMIN D RECEPTOR GENE POLYMORPHISM AND ITS SUSCEPTIBILITY WITH CORONARY ARTERY DISEASE

Ume Kulsoom[1], Sitwat Zehra[1], Tahir Saghir[2], Amber Khan[1], Syeda Nuzhat Nawab[1] and Abid Azhar[1]
[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan
[2]National Institute of Cardiovascular Diseases (NICVD), Karachi, Pakistan

Coronary artery disease (CAD) is one of the leading causes of mortality worldwide as well as in Pakistan. Vitamin D receptor (VDR) play crucial role in regulating the transcription of many genes and has significant impact on the morphology, proliferation, and growth of cardiac cells. The absence of VDR receptors, therefore, has many adverse effects on cardiac cells. The objectives of the study are to establish genotypic correlation by evaluating the vitamin D receptor gene variations in coronary artery disease patients and controls and to examine the role of VDR gene polymorphisms and its association with coronary artery disease. Blood samples of 150 coronary artery disease patients were included in the study. The samples were

compared with age and sex matched healthy controls. Genomic DNA was extracted. Genetic variations were analyzed through amplification-refractory mutation system-multiplex polymerase chain reaction (ARMS-Multiplex PCR) and results were examined by agarose gel electrophoresis. There are four types of VDR gene allelic polymorphisms which were observed in the samples both in cases and controls. The polymorphisms included are FokI, BsmI, TaqI and ApaI. The polymorphisms were of 77, 534, 148 and 229 (bp) DNA fragment respectively, which either exist as a monoallelic or biallelic forms. Polymorphisms encompasses the presence or absence of their respective DNA fragment referring as either wildtype, mutated one or both considering as heterozygous allelic variation in gene which were found in the coding region of the gene. This polymorphism in the promoter region of the VDR gene produces a non-functional receptor which may increase inflammation, leading to the enhanced progression of Coronary artery disease (CAD).

PURIFICATION, CHARACTERIZATION AND APPLICATION OF XYLANASE FROM *ASPERGILLUS NIGER* KIBGE-IB36

Urooj Javed[1], Fariha Ibrahim[1], Fehmina Khan[1], Afsheen Aman[1] and Shah Ali ul Qader[2]

[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

[2]Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan

Plant biomass has remarkable benefits of producing both products and energy. For comprehensive hydrolysis of lignocellulosic biomass, several enzymatic reactions are implicated. Xylanases are the enzymes that participate in the cleavage of internal β-(1,4)-linked D-xylosyl glycosidic bonds in hetroxylan to produce short xylooligosaccharides. In the current study, xylanase was purified using ultrafiltration gel permeation chromatography. To confirm the purity of enzyme, native PAGE gel and zymography was performed. The approximate molecular weight of purified xylanase was 110 kDa. The kinetic characteristics of purified xylanase showed optimal activity at 50°C with pH 5.0 within 10.0 minutes of reaction time. It was also observed that the purified enzyme showed stability with various metal ions, organic solvents and detergents. The relative amino acid composition showed greater amount of aspartic acid and N-terminal sequence of purified xylanase revealed that the enzyme has N-glycosylation properties that make the xylanase more stable and have high catalytic potential. In this study, biotechnological exploitation of xylanase for pre-treatment of agro-industrial wastes was performed that

showed efficient hydrolysis of wastes that produced fermentable sugars. Hence, the results obtained from the current study showed that the purified xylanase from *Aspergillus niger* KIBGE-IB36 have potential to be used in different industrial applications mainly in animal feed, paper and pulp industry and enzymatic pre-treatment of agro-industrial wastes.

MUTATIONAL ANALYSIS OF *TP53* GENE ASSOCIATED WITH ESOPHAGEAL CANCER IN BALUCHISTAN, PAKISTAN

Wafa Panezai[1], Naheed Sajjad[1] and Muhammad Naeem[2]
[1]Sardar Bahadur Khan Women's University, Quetta, Pakistan
[2] Quaid-i-Azam Unuversity, Islamabad, Pakistan

Cancer of the esophagus typically occurred in one of two forms, squamous cell carcinomas occurring in the stratified squamous epithelial lining of the organ, and adenocarcinomas affecting columnar glandular cells that replace the squamous epithelium. Tp53 gene is one of more important tumor suppressor gene, which acts as a potent transcription factor with fundamental role in the maintenance of genetic stability. The development of esophageal cancer is a multistep process resulting in successive accumulation of genetic alterations that culminates in the malignant transformation. The aim of the study was to find out the association of p53 gene with esophageal cancer in Baluchistan population, Pakistan and to screen out novel and reported mutations of Tp53 associated with esophageal cancer. Two hundred case and control blood samples were taken from tertiary care hospital, DNA from 100 ESCC patients and 100 controls

were taken out of which 20 patients and 20 controls were sequenced to detect tp53 mutations in exon 5 and 6. In both exons, mutations were not spotted only intronic mutations were detected in exon 6. With the help of T Personal Thermo-cycler genomic DNAs from 100 esophageal cancer patients and 100 controls was checked to detect deletions in Tp53 in exon 1, no deletion was found.

MOLECULAR ANALYSIS OF GLYCOSYLTRANFERASE GENES EFFECTED BY UV IRRADIATION IN *LEUCONOSTOC MESENTEROIDES*

Zahida Shabbir, Afsheen Aman, Fehmina Khan and Nadir Naveed Siddiqui
Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi-75270, Karachi, Pakistan

Dextransucrase and levansucrase enzymes are responsible for the production of commercially important biopolymers dextran and levan respectively, which possess favorable physiochemical properties with enormous industrial applications. Some of the *Leuconostoc mesenteroides* strains produced both enzymes and their biopolymers. In previous study by different doses of Ultra Violet irridiation (UV) mutations were induced in *Leuconostoc mesenteroides* (wild type) KIBGE-IB22 to enhance the yield of biopolymers. Among all different mutant strains, KIBGE-IB22M20 exhibited highest dextransucrase activity as compared to the wild type and the activity of levansucrase was completely suppressed. In the current study we are going to find out the effect of UV irridiation on respective genes. Effect of UV irradiation on dextransucrase gene of KIBGE-IB22M20 was studied

which confirmed that no mutation had occurred in dextransucrase gene despite of the fact production had increased. As dextransucrase gene did not display any mutation this give us insight into that due to suppression of competitor enzyme, consumption of substrate in dextransucrase was enhanced. Furthermore, levansucrase gene is amplified and is in process of sequencing. Sequencing may further help to find out the basis of suppression mechanism which may be beneficial for industrial purposes.

MICROBIAL ECOLOGY: THE ASSOCIATION OF CHICKEN GUT MICROBIOME AND BODY WEIGHT LINKED WITH DIETARY MODULATION

Zubia Rashid[1], Zulfiqar Ali Mirani[2], Arif Ali Chishti[1], Abid Azhar[1] and Saddia Galani[1]

[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

[2]Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi, Pakistan

Body weight is an economic trait in the poultry industry and might be associated with other variables. It is influenced by several genetic and environmental factors, such as diet, gut bacteria, immunity, rearing conditions, etc. For this, gut microbiota marks as key players by maintaining beneficial interactions with the host. An effective strategy for improved body weight gain is the identification and modulation of relevant bacteria through variation in the feed. The current study aimed to explore the association between targeted gut bacteria and weight gain in chickens through dietary changes. Quantitative analysis of bacterial genera including

Lactobacillus spp., *Enterococcus* spp., *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the chicken gut was performed employing molecular techniques. Also, performance parameters such as daily food intake, body weight gain and feed conversion ratio were measured up to 42 days. Chickens fed natural growth promoters diet revealed increased ($p<0.05$) body weight gain with lower food intake ($p<0.05$). Despite the role of gut microbiota in host health, studies based on the relationship between body weight gain and gut microbiota are scarce. Multiple linear regression analysis depicted *Lactobacillus* spp. and *Enterococcus* spp. were positively ($p<0.05$) and *Escherichia coli* and *Campylobacter* spp. were negatively correlated ($p<0.05$) to body weight gain. As a conclusion, the observed data of quantitative gut microbiota and its correlation with host weight showed that performance-related bacteria are positively correlated to body weight gain in broilers which deduce that dietary modulation is an effective strategy in poultry for desired results.