CHAPTER 54

ZOOLOGY

Doctoral Theses

01. AMIT
Development of Analytical Methods by Employing Chemometrics to Evaluate the Adulteration and Authenticity of Coconut Oil in India.
Supervisor: Prof. Dileep Kumar Singh
Th 25673

Abstract

Coconut oil is obtained from the fruit of the coconut tree (*Cocos nucifera*). Based on the extraction method, coconut oil is of two types i.e., virgin coconut oil (VCO) and refined, bleached, and deodorized (RBD) coconut oil. In recent times, virgin coconut oil (VCO) has rapidly become one of the most worthwhile edible oil after olive oil. This is not only attributed to its better fragrance and flavour but also its possible health advantages. VCO has a high proportion of medium-chain fatty acids like capric, caprylic, and caproic acids which are found to have antiviral and antimicrobial attributes. The adulteration of edible oils is a preeminent worry for the consumers and the oil-producing industries. Although the process of blending is based on profitable intention, the deed can adversely affect the quality of coconut oil. Mustard oil (MO), paraffin oil (PO), and palm oil are the prominent adulterants of VCO. While fried coconut oil (FCO) is one of the main adulterants blended in RBD coconut oil. In the present study, Fourier Transform Infrared (FTIR) spectroscopy, along with multivariate chemometrics, has been implemented for the detection and quantification of PO and MO in VCO, and FCO in pure RBD coconut oil (PCO) with great accuracy and precision. In addition to the adulteration detection, identification of the geographical origin of coconut oil is also one of the critical aspects for edible oil authenticity. To solve this issue, inductively coupled plasma mass spectrometry (ICP-MS) coupled with chemometrics has been used for the identification of the geographical origin of VCO samples of five provinces of India. Chemometrics included qualitative analysis (PCA, LDA) and quantitative analysis using regression modelling (PCR & PLS-R). Hence, the developed analytical methods can be used for the detection of coconut oil adulteration and authenticity in the field.

Contents

02. BALAN (Biji)
Physico-Chemical Characterization and Development of Analytical Method using FTIR Coupled with Chemometrics to Assess the Quality and Authenticity of Cow Milk.
Supervisor: Prof. Dileep Kumar Singh
Th 25661

Abstract

Cow milk is an excellent food source rich in fats, proteins, carbohydrates, vitamins, and minerals. It is the best and cheapest source of nutrition and is easily accepted by all age groups. Due to high nutritional values , milk consumption is increasing globally. Milk production is unable to fill the gap between supply and demand. To accomplish the linkage and to earn profits, milk is often adulterated by unethical producers. Composition of cow's milk is of great value for the dairy industry. The present study provides a comprehensive analysis of physio-chemical parameters and nutritional quality of cow milk. These parameters play an important role in monitoring the milk quality. The preliminary investigations showed that the milk compositions of all the samples were within recommended nutritional levels. These findings will be helpful for governmental authorities and public to monitor the milk quality. To limit the food fraud, continuous quality monitoring should be done. The present study uses Fourier transform infrared (FTIR) spectroscopy coupled with multivariate chemometrics as a quick quality monitoring method for the qualitative and quantitative analysis of formalin and sucrose adulteration in cow milk. Formalin has been added illegitimately to increase the shelf life of milk whereas sucrose is added to reconstitute milk's composition by improving the total solid contents. PCA and SIMCA were used as data reduction methods and classification methods. PLSR and PCR models were established for normal spectra, 1st derivative and 2nd derivative for the quantification of adulterants in milk. PLSR model for normal spectra showed the best prediction as compared to PCR models in both formalin and sucrose adulteration. This study indicates the FTIR spectroscopy coupled with chemometrics is quick and non-destructive analytical technique used for the detection and quantification of adulteration in milk. This approach constitutes a powerful alternative for the authentication of dairy products.

Contents

03. BHATT (Parul)
Study of the Immunogenicity and Adjuvanticity of DosR Regulon Proteins Rv2627c and Rv2628 of *Mycobacterium tuberculosis*.
Supervisors: Prof. Sadhna Sharma and Dr. Monika Sharma
Th 26526

Abstract

Tuberculosis (TB) remains a major public health problem worldwide and is among the top 10 infectious diseases. One-fourth of the world's population is already infected with latent tuberculosis (LTB). Ten million new cases and 1 million deaths due to TB were reported in 2019. Following primary infection, 5-10% of infected individuals develop active disease, and the remaining 90-95% of infected individuals either resolve or develop latent TB infection (LTBI). *Mycobacterium tuberculosis* (M.tb) persists in the latent stage for a long period without causing disease and acts as a reservoir that can serve as a new source of infection. The possibility of reactivation depends on the immunological balance between the

pathogen and the host, disruption in this balance results in the progression of LTBI to active disease. The rate of reactivation of latent TB is high in immune-compromised individuals suffering from HIV and diabetes. Therefore, latently infected individuals are at a greater risk of developing and transmitting TB. This enormous reservoir of latently infected individuals represents the main source of new TB cases. The current problem of TB can be combated by overcoming the drawbacks of the currently available BCG vaccine. This would involve the incorporation of antigens that can control TB at all stages including the dormant phase which is generally ignored. Hence, DosR regulon proteins, which are expressed in latent infection, could prove to be very good vaccine candidates as they can target the silent but most predominant form of TB infection. In the present study, the immune response to two DosR proteins Rv2627c and Rv2628 has been studied in PBMCs (Peripheral Blood Mononuclear Cells) derived from normal individuals, Category I Pulmonary TB patients (PTB), and healthy contacts of TB patients (HTB). It is found that these antigens were capable of stimulating a strong IFN-γ+ T cell response along with accentuation of memory T cells and other protective cytokines such as IL-2 and IL-17. At the same time, these proteins decrease the frequencies of immune-suppressor regulatory T cells in *in vitro* stimulation of PBMCs from both PTB and HTB. Considering all these facts together, we suggest Rv2627c and Rv2628 are extremely promising candidates for incorporation into a post-exposure subunit vaccine against TB. As T cells identify specific antigenic peptide residues presented via MHC class I and MHC class II molecules on their surface. The precision to trigger epitope-specific protective T-cell immune response can therefore be achieved with a synthetic peptidebased subunit vaccine. Therefore, in the second study, we have adopted an immunoinformatics approach to identify specific peptide residues from antigenic DosR regulon proteins that we have discovered to be strong T cell antigens. In our study, we have used ProPred, IEDB, NETMHC, BIMAS, Vaxijen2.0 for predicting immunogenicity and CABSDOCK, Hex 8.0, Pymol, Discovery Studio for docking and visualization to select 10 peptides from 4 latency-associated proteins (Rv2626c, Rv2627c, Rv2628, and Rv2032) of DosR regulon of M.tb. As Intracellular IFN-γ secreted by T cells is the most essential cytokine in Th1-mediated protective immunity, these peptides are verified as potential immunogenic epitopes in PBMCs of 10 HTB and 10 PTB individuals. The antigen-specific CD4+ and CD8+ T cells expressing intracellular IFN-γ are analyzed using monoclonal antibodies in all subjects by multi-parameter flow cytometry. Both PTB and HTB individuals responded to DosR peptides by showing an increased frequency of IFN-γ+CD4 and IFN-γ+CD8 T cells. The T-cell response is significantly higher in PTB patients in comparison to the HTB individuals. Additionally, our synthetic peptides and pools showed higher frequencies of IFN-γ+ CD4 and IFN-γ+CD8 T cells than the peptides of Ag85B. This pilot study can be taken up further in a larger sample size which may support the untapped opportunity of designing M.tb DosR inclusive peptide-based post-exposure subunit vaccine. Furthermore, subunit vaccines are required to be conjugated with adjuvant to increase their immunogenicity and now many TLR agonists are used as an adjuvant with these vaccine candidates. Only two such tuberculosis vaccine candidates ID93 and H56- IC31 having Rv1813c and Rv2660 latency antigens are under clinical trials. Future studies are required to find vaccine candidates targeting latent TB that will help in preventing TB reactivation. Adjuvants are essential in vaccine development because they improve vaccination immunogenicity by activating the innate and adaptive immune systems.

Contents

04. DAGAR (Vinay Singh)
Studies on the Growth Regulatory Effects of Emamectin Benzoate on the Cotton Bollworm, Helicoverpa armigera (Hübner).
Supervisor: Prof. Sarita Kumar
Th 26527

Abstract

Helicoverpa armigera, a global polyphagous pest, attacks, diverse crops causing huge agricultural loss. The current study evaluated the potential of Emamectin benzoate (EMB) against a laboratory-maintained population of H. armigera. The toxicity bioassay with EMB-dipped castor leaves and EMB-incorporated artificial diet resulted in the respective LD_{50} values of 0.245 µg/mL and 0.097 µg/mL Both the diets also caused considerable feeding deterrence in the larvae. Based on the results, thee EMB dosages, 0.01 µg/ML, 0.025 µg/mL and 0.05 µg/mL, with negligible/insignificant antifeedance and toxicity were selected for further investigations on the growth and development of larvae. The dietary EMB induced delayed development, reduced average weight gain in different developmental stages, diminished adult emergence, and decreased oviposition and egg hatch in H. armigera. The dietary EMB also caused a significant decline in the food digestion and assimilation. A noticeable increase in the total protein, while decrease in the carbohydrate and lipid reserves was recorded in these larvae. The dietary EMB also imparted distinct changes in the histoarchitecture of larval midgut with severely damaged and distorted epithelial cells, increased gut human and exfoliation of epithelium from the basement membrane. The EMB-diet fed larvae of H. armigera exhibited a significant reduction in the activity of gut transaminases, while it elevated the level if midgut phosphatases and detoxification enzymes; α-esterases, β-esterases, Glutathione-S-transferase, Acetylcholinesterase and microsomal oxidases (CYP450); in the larvae. The in-silico docking studies validated the interaction of all the seven midgut enzymes with the ligand EMB. The analysis of midgut protein profile through LC-MS in the EMB-fed H. armigera larvae identified differentially expressed 39 protein; 35 differentially deleted and 4 novel proteins; in comparison to the controls. These research outcomes suggest the potential efficacy of EMB to manage H. armigera and provide useful information in developing a strategy for its safe and effective management.

Contents

05. DHAULANIYA (Amit Singh)
Nutritional Characterization of Pure Indian Apple Juices and Development of FTIR based Chemometric Models for the Evaluation of Potential Added Sugar Adulterants.
Supervisor: Prof. Dileep Kumar Singh
Th 25704

Abstract

Our study involved twelve apple cultivars collected from the RHRTS, Mashobra, HP. For all the samples total soluble solids (TSS) content, acidic content (TA), total phenolics (TPH), and antioxidant activity (AOA) was measured. TSS and TA content in all samples ranged between 10.3±0.29 brix to 15.0±0.00 brix and 3.22±0.00 g/L to 5.36±0.00 g/L respectively. The TSS/TA ratio as a sweetness indicator of the samples was calculated. The highest TSS/TA ratio (39.06) was reported for Red delicious cultivars. An extraction method for TPH was also optimized. The best yield of TPH was obtained using 80% acetone-water mixture. The highest amount of TPH was estimated in the juice extracted from Red delicious cultivar (941.34±4ug ml^{-1}). Two different assays (DPPH and FRAP) were performed to check the AOA. The highest AOA was shown by red delicious and least was shown by top

red cultivar. Other than the biochemical analysis chemometric assisted FTIR models were developed for the estimation of added cane sugar and corn syrup in apple juices. From primary analysis fingerprint region in the range of 1200cm^{-1} to 900cm^{-1} was identified. PCA helped us to reduce our data set in terms of variables. In case of cane sugar SIMCA shoed 100% classification efficiency (Raw data set), and LDA was able to classify the test set with an accuracy of only 96.67%. For quantitative prediction of cane sugarPLS-1st derivate (R^2 = 0.991) model was found best optimized against all models. In case of corn syrup the SIMCA and LDA both performed equally well with classification efficiency of 100% for quantitative prediction PPLS model (R^2 = 0.999) for raw data set showed better results as compared to other models. In conclusion, FTR based chemometric models can be considered as reliable methods for addressing the problem related to sugar adulterations in apple juice.

Contents

06. GANGWAL (Aakriti)
Deciphering the Role of a Novel Serine/Threonine Phosphatase in the Life Cycle of Bacillus Anthracis.
Supervisor: Prof. Yogendra Singh
Th 25652

Abstract

Reversible protein phosphorylation at serine/threonine residues is one of the most common protein modifications, widely observed in all kingdoms of life. The catalysts controlling this modification are specific serine/threonine kinases and phosphatases that modulate various cellular pathways ranging from growth to cellular death. Genome sequencing and various omics studies have led to the identification of numerous serine/threonine kinases and cognate phosphatases, yet the physiological relevance of many of these proteins remain enigmatic. The study in this thesis is focused on the functional characterization of another ser/thr phosphatase (PrpN) in *Bacillus anthracis* and with the aid of a null mutant strain (BAS Δ*prpN*) we provide important insight regarding the role of PrpN in the life cycle of *Bacillus anthracis*.

Contents

07. JAMWAL (Rahul)
Development of Analytical Methods Using Chemometrics to Assess the Authenticity, Quality and Safety of Mustard Oil in India.
Supervisor: Prof. Dileep K. Singh
Th 25653

Abstract

Edible oils play an essential role in our routine life as cooking or frying oil as well as an ingredient used in food, medicine, and cosmetic commodities. Currently, the consumption of vegetable oils rich in mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids PUFA are in consumer's demand because of their concern for a balanced and healthy diet. Mustard oil (MO), is an elementary component of the Indian diet, as it increases the good HDL cholesterol ratio, and omega-3 and omega-6 fatty acids lower the risk of cancer. The significance of MO is generally attributed to its antibacterial, antifungal, and anti-carcinogenic properties, high content of oleic acid (increase shelf life of oil), natural antioxidants, tocopherol, phytosterols, vitamin K and polyphenols. Because of the high consumption demands of edible oils, adulteration incidents have immensely risen. Thus, adulteration detection is very crucial for consumers, oil-producing industries, and regulatory authorities. The act of forgery can be either accidental or purposefully that involves high-priced oil adulterated with low price edible or non-edible oils and can have great economic, social, and public health repercussions. The traditional analytical methods are usually time-taking, tedious, detrimental, lacking online monitoring, and need extensive sample preparation. Therefore, for the present study we have used Fourier transform infrared (FTIR) spectroscopy which is a excellent technique for the detection of edible oil adulteration. It utilizes the fingerprint region of the obtained spectra to differentiate and detect the different adulterants present in the edible oil. For the detection of Argemone oil (AO) adulteration in Mustard oil (MO) we have applied ATR-FTIR spectroscopy along with multivariate chemometrics. From Principal Component Analysis (PCA), through spectral regions 3050-2750 cm-1 and 1800-500 cm-1, we observed well-defined discrimination of MO from AO adulterants. Linear Discriminant Analysis (LDA) was also successfully applied to classify MO from AO. For quantification, Principal Component Regression (PCR) and Partial Least Square Regression (PLS-R) regression models were developed using combined optimized spectral regions (3050-2750 cm-1 and 1800-500 cm-1), and two separate optimized regions, 3050-2750 cm-1 and 1800-500 cm-1 respectively for normal, 1st, and 2nd derivatives. PLS-R model for 1st derivative spectra region of 1800-500 cm-1 showed best calibration model, with high precision and accuracy based on the values of Residual Predictive Deviation (RPD) of 52.23, Relative Prediction Error (RE %) of 0.033, Coefficient of Determination (R2) of 0.999 and Root Mean Square Error of Prediction (RMSEP) of 0.2. Likewise, MO adulteration with Linseed oil (LSO) was also analyzed using ATR-FTIR spectroscopy. Based on spectral information of adulterated samples, exploratory methods such as PCA and classification method like LDA was employed that correctly classified adulterated samples, providing an accurate classification of 100%. Furthermore, quantitative methods like PCR and PLSR were used to compare raw, first, and second derivatives of three selected optimized spectral regions to obtain the best model. The PLSR model for the first derivative of optimized spectral region II (1800 to 600 cm-1) showed excellent precision and accuracy ability in predicting adulterated samples with high R2 (0.999), RPD (63.42), and low standard error values (RMSECV; 0.216 v/v, RMSEP; 0.167 v/v and RE%; 3.45). Therefore, our results showed that the optimized statistical model has the potential to rapidly detect AO and LSO adulteration in MO as low as 0.5% v/v and 1% v/v. MO deterioration during the deep-frying process is also one of the major safety concerns related to deep-fried food products. Adulteration of Pure Mustard Oil (PMO) with used Fried Mustard Oil (FMO) causes potential harm to the health of the consumers due to lack of knowledge related to harmful effects of used FMO by local street vendors and restaurants. Therefore, it is important to develop a rapid method for the detection of used fried oil in fresh edible oil to examine oil quality. ATR -FTIR spectroscopy integrated with chemometrics was effectively applied for the rapid detection and accurate quantification of Fried Mustard Oil (FMO) adulteration in Pure Mustard Oil (PMO). PCA elucidated the studied adulteration using two components with an explained variance of 97 %. The LDA was adopted to classify the adulterated PMO samples with FMO. LDA model showed 100% accuracy initially, as well as when cross-validated. To enhance the overall quality of models, characteristic spectral regions were optimized, and PCR and PLS-R models were constructed with high accuracy and precision. PLS-R model for the 2nd derivative of the optimized spectral region 1260-1080 cm-1 showed best results for prediction sample sets in terms of high R2 and RPD value of 0.999 and 31.91 with low RMSE and RE % of 0.53 % v/v and 3.37 %, respectively. Thus, the suggested method can detect up to 0.5% v/v of adulterated FMO in PMO in a short time interval. The food products are usually mislabeled about their origin and are adulterated by adding cheaper products which affect the required standard of the agricultural produce, the credibility of the producers and traders. Various analytical approaches have

been used successfully to monitor the geographical origin of the food, as they offer fast and effective results compared to conventional approaches and do not involve reagents, a fact that favors the environment. Inductively coupled plasma-mass spectrometry (ICP-MS) is a preferred tool for geographical origin studies as compared to others due to its high selectivity and sensitivity. Using the multi-elemental profiles of mustard oil samples, we have identified 11 elements suitable for discriminating mustard oil samples from various provinces as well as for tracing their geographical origin. Classification methods such as PCA and HCA were found very useful for the initial selection of major elements. Linear discriminant analysis of the samples from different provinces provided a valid prediction model with 100% accuracy. Therefore, the present demonstrated the suitability of the FTIR and ICP-MS technique as a quality control tool for rapid and swift detection of adulteration in Mustard oil. Apart from being non-destructive in nature, these techniques also allow simultaneous measurement of a variety of components. However, the developed models should be verified with a larger data set that particularly includes the samples of vegetable oils from different/unknown origins.

Contents

08. JAMWAL (Rohit)

Characterization of Groundnut Bud Necrosis Virus (GBNV) and Studying the Interaction of Virus Glycoprotein (G_N) with Thrips Palmi.

Supervisor: Prof. Rajagopal Raman

Th 25671

Abstract

Groundnut bud necrosis virus (GBNV) is a tripartite ambisense single stranded RNA virus that belongs to the genus *Orthotospovirus.* GBNV infects many plant species and is transmitted by its insect vector *Thrips palmi*. The virus consists of 3 RNA segments S, M and L which help in virus replication and transmission. Viral Msegment encodes two glycoproteins, which encase the viral surface. GN and GC are the key genetic determinants in virus acquisition and transmission. The virus receptors and other proteins in the thrips midgut that interact with virus GN protein are still not known. Hence understanding the nature of virus proteins and identifying the proteins in insect vector that interact with virus GN is of prime importance. In the first objective we successfully amplified, cloned and expressed the GBNV GN and GC, N, NSm and NSs genes. We successfully purified these proteins and raised the polyclonal antibodies in mice. We also analysed the virus protein sequences by using various software. We constructed and validated the homology based 3D model for GN and N protein by using SWISS-MODEL softwares. In the second objective, we performed yeast-two hybrid assay to identify the potential interacting proteins of *Thrips palmi* midgut with the GBNV glycoprotein GN. We found two promising proteins, cathepsin-L and clathrin heavy chain that showed interaction with GN. Interactions of cathepsin-L and clathrin heavy chain with GN were validated by employing In-vitro pull-down assay and dot-blot assay. Results have suggested that these proteins are important in internalizing and stabilizing the virus within the midgut of thrips. Hence our present study has contributed towards a collective effort in understanding GBNV proteins and recognition of novel orthotospoviral receptors and interacting proteins in its insect host.

Contents

09. KHANNA (Anoushka)
Novel Strategies for the Management of Hematopoietic form of Acute Radiation Syndrome in Experimental Models.
Supervisors: Prof. Rina Chakrabarti and Dr. I. Prem Kumar
Th 25651

Abstract

The occurrence of a nuclear or a radiological emergency is uncommon with a very low reproducibility rate but is a matter of grave concern. Mankind exploits radioactivity for its need but is not well equipped to handle an emergency radioactive fallout. Radiation is frightening and creates panic as it cannot be seen or sensed in any way. Radiation accident is defined as an event involving the release of radioactive materials that has led to a serious impact on people, the facility or, the environment. The rise in the possibility of a radiological accident is due to the enhanced terrorist activities, industrial use, growing use of radiation therapy, usage of radioisotopes in medicine, transport of radioactive material and increase in global nuclear generation. Apart from the global health concerns, radiation accident or a planned terrorist attack is also accompanied by financial as well as mental stress. Besides the intended use of ionizing radiation, the risk of accidental exposures during radiation therapy, can at times have devastating health consequences which may even be more dangerous when it goes unnoticed. HSCs are the principal regulator of hematopoiesis which differentiate progenitors which further gives rise to differentiated and mature lineages. The loss of HSCs due to radiation leads to inability of the system to generate the differentiated lineages which ultimately results in hematopoietic syndrome. Hematopoietic form of acute radiation syndrome is an important one leading to compromised immune status and death due to opportunistic infections. In spite of more than six decades of research in the area of radiation countermeasures, currently there is no molecule which is safe and effective for human applications. Moreover, Bone marrow transplantation has been understood to be essential for the management of radiation induced hematopoietic syndrome. For this reason, both allogenic and autologous HSCT have been established as more or less noncontroversial strategies for the management of a hematopoietic malignancies and planned chemotherapy induced bone marrow depression. HSC's ability to self-renew and also to differentiate into hematopoietic lineages, render them indispensable for HSCT. Umbilical cord blood is a rich source of HSCs and is of great use to treat hematological disorders, (malignant and non-malignant) where HSCT is inevitable. The inadequate number of HSCs per unit cord blood (CB) is a major hindrance to their use. The proper homing of HSCs in the bone marrow niche leads to improved engraftment efficacy even with limited number of HSCs, thereby circumventing the need for *ex-vivo* HSC expansion. For successful transplantation of HSCs, it is quite necessary that efficient homing, engraftment and retention of HSC self-renewal capacity takes place, which is often restricted due to inadequate number of adult HSCs. Apart from this, HSCs are known to reside in hypoxic niches inside the bone marrow and hematopoietic transition from hypoxic bone marrow niches to non-physiological ambient air during HSC collection leads to ROS production and opening of mitochondrial permeability transition pore, causing poor HSC survival and loss of their long-term repopulating potential. Additionally, interaction of radiation with biological system leads to the generation of free radicals (ROS, RNS) which further interacts with the macromolecules and results in severe damage due to oxidative stress. Use of antioxidants as radioprotectants (administered prior to radiation) have been reported to be valuable in treating radiation damage. So, in view of this, small molecule libraries were screened for potential MPTP inhibitors and NaHS (H2S donor) was identified as one of the potent inhibitors.

Contents

10. MANMOHAN KUMAR
Role of TLR22 in Bacterial Pathogenesis in Fish.
Supervisors: Prof. Umesh Rai and Prof. Shibnath Mazumder
Th 25897

Abstract

Organisms survive in a potentially unreceptive environment enclosed with a mystifying array of infectious agents of diverse size, shape, composition and rebellious character, which keep on clashing with their hosts to utilize them as rich reservoirs for promulgating their 'selfish genes'. Therefore, natural selection acted strongly on the vertebrates' defense system to counteract against microbial trespassers, particularly on the innate immune system. The innate immune system consists of germline-encoded immune sensors which scrutinize these non-self-rebellions from hosts' self-molecules and thus license the interdiction of infection before proliferation and dissemination of microbes, and overwhelm the host. A vast array of host innate immunity receptors known as pattern-recognition receptors (PRRs) exist to achieve nonstop surveillance of intruding pathogens. These PRRs senses the unique motifs referred to as pathogen-associated molecular patterns (PAMPs) present in viruses, bacteria, and fungi. These sensors act as the frontline soldiers in innate immune response against these microbes and are anticipated to be a prime target of natural selection in species adapted to different habitats with discrete pathogenic load. Toll-like receptors (TLRs) are the extensively studied immune sensors amongst the various PRRs and over the last few years, compelling reports addressed the key advances in the understanding of TLR-mediated immunity in the vertebrates. The constant exposure of pathogens and the distinct habitat shaped the fish immune system and evolved TLR family which led to the expansion of novel TLRs. In recent years, studies have shown few components of the microbes as the ligands of TLRs and their specificity has been proven for TLRs which is limited to mammalian TLRs only. TLR22 is one of the representative members of novel non-mammalian TLRs present in fish (Ding et al., 2018). It has been reported in several fish species. Earlier, TLR22 was reported to be a dsRNA virus receptor and a functional substitute for TLR3 (Matsuo et al., 2008) and recently it has been recognized as a crucial sensor in innate immune signalling pathway against parasitic and bacterial infection as well (Panda et al., 2014; Li et al., 2017). This receptor has been reported at immune and nonimmune sites suggesting its functional plurality. Although, the comprehensive evidence about TLR22 localization, ligand recognition and immune signalling cascade is lacking in fish. Therefore, the present study was aimed at reducing the role of TLR22 in bacterial infection in fish. Introduction Chapter 1 2 The eukaryotic cell is a complex milieu in which essential tasks such as protein synthesis, internalization of external solutes, and storage of genetic information are partitioned in membrane-bound structures. From the viewpoint of an intracellular bacteria, the eukaryotic cell might be considered as a rich pool of nutrients as well as a sheltered environment in which bacterial replication may take place, avoiding contact with extracellular host defenses such as antibodies or complement. Therefore, the involvement of TLR22 was studied at the cellular level using headkidney macrophages (HKM) from Clarias gariepinus as the host cells

and Aeromonas hydrophila as the bacterial pathogen. Earlier reports from our laboratory have established HKM as an invitro model to study host-pathogen interaction (Banerjee et al., 2014; Shelly et al., 2017; Datta et al., 2018; Hussain et al., 2019). The role of TLR22 in the interaction between A. hydrophila and HKM was explored which can address the key cellular and molecular events crucial for TLR22-signalling in regulating bacterial pathogenesis and designing effective strategies for its control.

Contents

1. Introduction 2. Review of literature 3. Objectives 4. Objectives 1[to study the involvement of TLR22 in HKM death in bacterial infection] 5. Objectives 2[to elucidate the role of TLR22 in activation of extrinsic cell death pathway in bacterial infection] 6. Objective 3 [to elucidate the role of TLR22 in activation of intrinsic cell death pathway in bacterial infection] 7. Summary, Future work 8. Material and methods. References and List of publications.

11. MEDHA
Understanding of the Role of Mitochondria in Inducing Macrophage Apoptosis in Response to Antigenic Proteins of Mycobacterium Tuberculosis.
Supervisors: Prof. Sadhna Sharma and Dr. Monika Sharma
Th 25672

Contents

Objective 1: To study the role of Rv2615c (PE_PGRS45) and Rv0335c (PE6) in targeting host mitochondria and disrupting mitochondrial integrity 1. Cloning, expression and purification of Rv2615c and Rv0335c 2. Role of Rv2615c and Rv0335c in targeting and disrupting host mitochondrial integrity Objective 2: To study the role of Rv2615c (PE_PGRS45) and Rv0335c (PE6) in inducing host macrophage apoptosis and to see whether it is TLR dependent 3. Role of Rv2615c and Rv0335c in induction of host macrophage apoptosis 4. To study the TLR dependent mechanism of Rv2615c and Rv0335c medicated macrophage apoptosis Objective 3, 5. Role of C-terminal domain of PE6 (Rv0335c) protein in inducing host mitochondrial stress and macrophage apoptosis. Summary. Publications and conferences.

12. MEHTA (Priyanka)
Study the Role of miRNAs Involved in the Pathogenesis Induced by M. fortuitum in Kidney Macrophages of Zebrafish.
Supervisors: Prof. Umesh Rai and Prof. Shibnath Mazumder
Th 25662

Abstract

Mycobacterium fortuitum has been extensively used to understand mycobacterial biochemistry and for large scale screening and development of anti-tubercular drugs. However, not much research has been done on the pathogenesis of *M. fortuitum*. A complete understanding of mycobacteriosis requires standard model systems. There are only few model systems available to study the pathogenicity of *M. fortuitum* in fish, the natural host for the bacteria. Thus, we have developed alternate animal model zebrafish (*Danio. rerio*) to study the pathogenesis of *M. fortuitum in vitro*. The present study aimed to study the pathogenesis caused by *M. fortuitum* at cellular level in ZFKM (zebrafish kidney macrophages) to understand the progression of disease and to get a better understanding of innate and adaptive immune responses generated against bacterium. We also sought to determine the role of miR-155 and miR-146a in the pathogenesis and cell survival during infection. Although *M. fortuitum*

exhibits wide host range but there is dearth of studies involving miRNAs in *M. fortuitum* induced pathogenesis. Thus, understanding the underlying mechanisms of pathogenesis is of supreme importance not only because of its zoonotic importance but also due to increased reports from immunocompromised individuals. With our experiments we have tried to the unravel the microRNAs and their pathways involved in *D. rerio*. *M. fortuitum* infection in ZFKM induces up-regulation of miR-155 and miR-146a in TLR-2 and NF-κB dependent manner. Inhibiting TLR-2, PI3K and NF-κB inhibits the induction of both miR-155 and miR-146a. *M. fortuitum* induces ZFKM apoptosis which is amplified in the presence of miR-155 mimic and declined in the presence of miR-146a. Both these miRNAs act in opposite manner. Using combination of miRNA specific mimic and inhibitor we established that miR-155 induces pro-inflammatory response (TNF-α and IFN-γ) and M1 (IL-1β, IL-12 and iNOS) phenotype of macrophages by activating T-bet/Stat1 signalling and thus leads to bacterial clearance. Additionally, miR-155 acts on SOCS1, an established target and in turn promotes Stat1 signalling. miR-146a on the other hand, augments anti-inflammatory responses (IL-10 and IL-4) and promotes M2 phenotype (TGF-β) thus leading to survival of mycobacteria inside macrophages by targeting IRAK-1 and Traf-6 by hampering iNOS production. Both, these miRNAs fine tune the immune response for appropriate control of essential immune functions by inducing appropriate Transcription factors. We here hypothesise induction of miR-155 in response to *M. fortuitum* infection, and thus induced pro-inflammatory response initiated a negative feedback cascade which induces the expression of miR-146a to control the detrimental effects of induced pro-inflammatory response.

Contents

13. MITTAL (Disha)

Redox-Resetting of Cisplatin-Resistant Ovarian Cancer Cells by Cisplatin Encapsulated Nanostructured-Lipid Carriers (CisNLC); in Vivo Pharmacokinetics and Toxicity.

Supervisor: Prof. Anita Kamra Verma

Th 25656

Abstract

Cancer is a disease cluster characterized by unregulated replication leading to cells that become unhealthy. Cancer was first reported around 1600 BC in an Egyptian papyrus, and it was known until the 19th century to be an incurable condition. It is one of the leading causes of death worldwide and accounted for an estimated 9.6 million deaths in 2018 worldwide. As per the Indian Council of Medical Research (ICMR) data, India had 1.4 million cancer patients in 2018 and this number is expected to increase further. Ovarian cancer is the 7th most common cancer in the world, and the 8th most common cause of cancer death among women. The Globocan report reported that there were 239,000 cases and 152,000 deaths in 2012 (representing 3.6% of cancer cases and 4.3% of cancer deaths). Worldwide, nearly 600,000 people are diagnosed with ovarian cancer within five years (5-year prevalence). The Globocan report estimates that there will be a 55 percent rise in prevalence worldwide to 371,000 by 2035 and a 67 percent increase in deaths to 254,000 by 2035. It brings a projected lifetime risk of one in 54 to 75 and one in 100 mortality associated with ovarian cancer. The age-standardized prevalence in developed countries is about 9.4 per 100 000 and 5 per 100 000 in less developed areas. Symptoms may be ambiguous and often misattributed to irritable bowel syndrome, sometimes diagnosed at an advanced level. Epithelial Ovarian cancer (EOC) ranks mostly as 3rd or 4th most common cancer in India. The first two leading sites of cancers being breast and cervix. The three main treatment options for cancer patients are surgery, chemotherapy, and radiation therapy. In most cases, however, a combination of

surgical and chemotherapeutic treatments is utilized. Chemotherapeutic treatments in general lack specificity because they target all proliferating cells by inhibiting DNA synthesis or interfering with processes of cell division or metabolism. As a consequence, chemotherapy leads to the damage of healthy tissue, especially of the normally dividing cells of the bone marrow, skin, and gastro-intestinal mucosa, among other tissues. The poor specificity of chemotherapeutic agents commonly prevents aggressive and effective treatment of the cancer. Development of therapeutic resistance limits the efficacy of current cancer treatment. Understanding the molecular basis for therapeutic resistance should facilitate the identification of actionable targets and development of new combination therapies for cancer patients. Yet the understanding of therapeutic resistance still remains incomplete. Toxicity due to off-target effects and the development of resistance have compromised the effectiveness of chemotherapies. Hence, there is an urgent need for alternate better-targeted approaches Hence, novel strategies and therapeutic molecules against ovarian cancer urgently need to be investigated. Cisplatin is one of the most active single agents against ovarian carcinoma, with a response rate of 50-65% in previously untreated patients. For the last 4 decades, cisplatin has been the most active ovarian cancer chemotherapy medication, and the prognosis for women with ovarian cancer can be determined by the tumor reaction to cisplatin. Many patients with innate platinum-resistance have very low prognosis. Although, most ovarian cancer patients respond to front-line platinum combination chemotherapy, many become unresponsive to cisplatin and eventually the patient succumbs to the disease. Cisplatin is the most widely used drug for ovarian cancer, but its use is limited owing to some significant side effects in normal tissues, the use of cisplatin is restricted, such as nephrotoxicity, ototoxicity, neurotoxicity, gastrointestinal toxicity and haematological toxicity. Also, side effects such as cardiotoxicity, hepatotoxicity, retinal toxicity, syndrome of inappropriate antidiuretic hormone. Although the responsiveness of Cisplatin in the initial stages of EOC is higher but the problem of development of resistance arises which causes the relapse of EOC. Cisplatin is a single platinum atom, coordinated with two amide groups and two reactive chlorides. The comparatively low intracellular chloride concentration intracellularly drives the substitution by water of one or both of the chloride moieties, resulting in positively charged species that react to cross-link DNA, RNA, and protein with nucleophilic sites. The levels of RNA and protein binding are poor at pharmacologically relevant doses and cisplatin cytotoxicity tends to coincide with the development of DNA adducts. The mechanism of resistance is divided into two broad categories: 1) those limit the formation of cytotoxic platinum-DNA adducts and 2) those that prevent the cell death occurring after the platinum-DNA adduct formation. Development of therapeutic resistance limits the efficacy of current cancer treatment. Understanding the molecular basis for therapeutic resistance should facilitate the identification of actionable targets and development of new combination therapies for cancer patients. Yet the understanding of therapeutic resistance still remains incomplete. Toxicity due to off-target effects and the development of resistance have compromised the effectiveness of chemotherapies. Hence, there is an urgent need for alternate better-targeted approaches. To enhance the half-life of Cisplatin, prevent Cisplatin induced toxicities suitable delivery systems are necessary. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Nanoparticles are functionally active owing to their large surface to volume ratio. Nanomedicine, which applies nanotechnology to highly specialised medical interventions for disease prevention, diagnosis and treatment, is one of the most involved research fields of nanotechnology. Nanostructured Lipid Carriers (NLC) are second generation of Lipid nanoparticles which are a blend of solid and liquid lipid which at room temperature remains solid and avoids the use of organic solvents. NLCs have advantages over Liposomes and polymeric nanoparticles as they can entrap a high amounts of drug, can improve drug stability, have the ability to regulate the release of drugs and surface can be easily modified with suitable targets. The work involves engineering biosafe Cisplatin encapsulated NLC (CisNLC), along with its characterization. CisNLC was synthesized by ultrasonication method and the physical characterization of NLC and CisNLC by DLS, TEM, SEM-EDX, DSC XRD, FTIR was done. Unimodal distribution of nanoparticles was evident by DLS. The hydrodynamic size of CisNLC and NLC evaluated by DLS were found to be 141.5 nm (PDI 0.249) and 111 nm (PDI 0.238) respectively. The electrophoretic mobility and surface charge of nanoparticles were measured by the frequency shift of the scattered light at a 12° scattering angle and was -41.5 ±1.62 mV. The percentage entrapment efficiciency (% EE) of prepared CisNLC was ~72.75%, determined by UV-Vis spectroscopic method and confirmed by SEM-EDX. Further, we synthesized Rhodamine B loaded NLC to study the cellular uptake mechanisms. The Electron microscopy (TEM) studies indicated the monodispersity and

spherical shape of the nanoparticles in the range of ~ 130 nm. Images from TEM are two-dimensional sections of the material under study and were in unison with the DLS data. The transmission FTIR spectrum shows the characteristic peaks of Cis were absorbed at 3281 cm−1 (−NH stretch), 3204.6 cm−1 (−OH stretch), 1630 c.m−1 (C=C stretch), 1296 cm−1 (C-O stretch). Precirol identification peaks were identified at 1731 cm−1 (C-O stretch), 2916.3 cm−1 (C-H stretching) along with free –CH3 group at 2819 cm−1 and –CH2CH2 group at 1469 cm−1. All the characteristic peak of Precirol are present in both NLC and CisNLC. XRD studies indicated low intensity peak of cisplatin in CisNLC diffractogram. DSC studies complete encapsulation of cisplatin in CisNLC lipid matrix. The release kinetics of the NLC and CisNLC was done at pH 7.4 and pH 5.8. It was observed that release from CisNLC followed a controlled first order, Fickian diffusion pattern at physiological pH 7.4 and controlled first order release mechanism at pH 5.8 The release pattern of cisplatin from CisNLC was burst release in the initial stages followed by sustained release. The therapeutic efficacy of CisNLC *in vitro* along with the sensitization of CaOV3 cells towards cisplatin. For understanding the internalization of nanoparticles cellular uptake was assessed in a fluorescence microscope. PA-1 and CaOV3 cells were treated with Rhodamine B loaded NLCs. The cells were fixed and visualized in a fluorescence microscope (Eclips 90i, Nikon). They appeared to be internalized and deported to the cytoplasm and the nuclei were counterstained with DAPI. To assess the biocompatibility of NLC, Cis *per se* and CisNLC on PA-1and CaOV3 cells by the colorimetric MTT cell viability assay. It involves the reduction of yellow coloured MTT into purple coloured formazan crystals that was proportional to the cellular metabolic activities due to NAD(P)H-dependent cellular oxido-reductase enzymes in living cells. The IC50 value (µg/mL) of NLC, Cis per se and CisNLC GNP and EGNP post 24hr of treatment against MCF-7 and HEK-293 cells at 37 °C under 5% CO2 were assessed. The IC50 value (µg/mL) of NLC, Cis and CisNLC post 24hr was 8016.37 µg/mL, 111 µg/mL, 210 µg/mL in PA-1 cells and 21154.33 µg/mL, 205 µg/mL and 500 µg/mLin CaOV3 cells, respectively. Cellular ROS levels can be quantified in live cells by DCFDA assay and it proves that CisNLC has caused generation of H2O2 in both PA-1 and CaOV3. Moreover, biotoxicity of NLC, Cis *per se* and CisNLC was assessed on PA-1 and CaOV3 cells by estimating the level of LDH and RNS. The growth-inhibitory effects of therapy determined by LDH and NO assay were supportive with the results determined by MTT assay. To elucidate the effect of CisNLC on the redox resetting of CaOV3, it was imperative to assess the role of Glutathione and related enzymes on the levels of ROS and cisplatin resistance markers leading to induction of cell death and finally its acute toxicity and oxidative stress in *in-vivo* mouse model. Although the mechanism of sensitization of CaOV3 by CisNLC is ambiguous, various biochemical alterations can be attributed to generation of oxidative stress. Therefore, the antioxidant enzyme assay (GSH, GP, GR, SOD) were evaluated in both PA-1 and CaOV3 cells. Since, induction of ER stress is a critical marker and is a potential target in cancer treatment, it was imperative to elucidate the interplay of ROS generation and sensitization of CaOV3 towards Cis. Intracellular Ca2+ concentrations were assessed by Flou-4AM assay. The increase of Ca2+ elicited by CisNLC could be because of their rapid destruction due to their translocation through lysosomes and/or endosomes in cancer cells during intracellular activities which are rich in the number of hydrolases in the acidic environment. Maximum fragmentation of DNA was observed with CisNLC treated cells, when compared with NLC and Cis *per se* treated cells. We have further elucidated the modulation of Cisplatin resistance markers i.e ATP7B and GSTP1induced by the treatment of CisNLC using RTPCR. Along with this the mode of cell death was also assessed by the expression of Bax, Bcl2 and Cas 9. The effect of CisNLCs on proliferation and sensitization of Cisplatin reistant ovarian cancer cell (CaOV3) towards cisplatin was significant. Its cellular uptake in CaOV3 and PA-1 were reported to be interesting. Biocompatibility of NLC have been established by the negligible hemolysis observed at the selected dosages. Pharmacokinetic properties of Cis *per se* in comparison to CisNLC were studied incdicating the improvement in the pharmacokinetic properties of cisplatin when encapsulated in NLC. Biodistribution studies of RhoNLC indicated maximum accumulation in spleen in comparison to other organs. Acute toxicity and oxidative stress of NLC, Cis *per se* and CisNLC in Balb/c mice model were assessed. Once a week intra venous injections of 10 mg/kg mice body weight of NLC, Cis *per se* and CisNLC) upto 14 days. Acute toxicity assay involves the assessment of ALT, AST, ALP, Urea and Creatinine in serum which proved that there was negligible toxicity by the treatment of CisNLC in comparison to Cisplatin alone. Oxidative stress involves the assessment of LDH, GSH, GP, GR and SOD in liver kidney, spleen, heart and lung. The toxic effects were then further confirmed by the histopathology of the vital organs i.e. liver kidney, spleen, heart and lung indicated that CisNLC generated minimal oxidative stress in comparison to cisplatin alone. The use of *in vitro* models helped

in assessing the efficacy with a mechanistic approach allowed much smaller quantities of chemicals for testing, but *in vivo* studies are required to support *in vitro* results. The current study could be applied in *in vivo* models to be applied for therapeutic purposes for combating the problem of cisplatin resistant in ovarian cancer patients.

Contents

1. Introduction 2. Review of literature 3. Material and methods 4. Synthesis and characterization of nanostructured lipid carriers 5. In vivo release kinetic studies 6. To determine the therapeutic efficacy in vitro 7. Ex vivo biocompatibility, pharmacokinetics & biodistribution of nanostructured lipid carriers 8. In vivo: Acute toxicity studies and oxidative stress 9. Discussion. Summary. References. List of publications and posters presented.

14. MONIKA

Exploring the Hexachlorocyclohexane (HCH) Isomers Specific Responses in an Archetypical HCH Degrader Sphingobium Indicum B90A and Sphingopyxis sp. MC4.

Supervisors: Prof. Ram Krishan Negi and Prof. Rup Lal (Retd.)

Th 25659

Abstract

The biosurfactant produced by strain MC4 is either lipoprotein or lipopeptide nature which has not been reported earlier in *Sphingopyxis* sp. The biosurfactant is stable at high temperature, salinity and acidic conditions which is advantageous for its application in harsh environmental conditions. Strain MC4 able to produce the biosurfactant by utilising various carbon sources in minimal media which is beneficial from economical point of view for large scale production. The interesting property of the biosurfactant is that it stabilise the hydrophobic pesticide HCH in aqueous media hence increases the availability of HCH for degradation. Overall the biosurfactant produced by strain MC4 can fuel up the process of bioremediation by applying it in the HCH contaminated sites. The detailed structural characterization of the biosurfactant is required in order to understand the interactions with hydrophobic pesticide.

Contents

1. Introduction 2. Review of literature 3. Comparative proteome analysis of an archetypical HCH degrader sphingobium indicum B90A in response to different HCH isomers 4. Evaluation of hexachlorocyclohexane biodegradation potential of sphingopyxis sp. MC4 isolated from HCH contaminated soil 5. Isolation and characterization of biosurfactant produced by sphingopyxis sp. MC4 and its potential application in HCH biodegradation. References. Appendices. List of publications. Conferences.

15. MUNENDRA KUMAR

Molecular Analyses of Extracellular Chitinases and Antifungal Compounds from Streptomyces Spp.

Supervisor: Prof. Monisha Khanna Kapur

Th 25670

Contents

1. Introduction 2. Review of literature 3. Screening of bioactive potential in Streptomyces spp. (colonies 130, 169, 194 and 165) 4. Genome mining of Streptomyces sp. strain 130 for the analysis of extracellular chitinase producing genes and biosynthetic gene clusters 5. Evaluation of antifungal activity of bioactive

compounds from Streptomyces sp. strain 130 6. Molecular and in silico studies of chitinases from Streptomyces sp. strain 130. Summary. References. Annexure and List of publication.

16. NAGAR (Shekhar)
Exploring the Microbial Diversity and Functional Dynamics of a Meso-Thermic Himalayan Hot Spring Khirganga, Indian Using (Meta) Genomics.
Supervisors: Prof. Ram Krishan Negi and Prof. Rup Lal (Retd.)
Th 25654

Abstract

Sulfur Related Prokaryotes (SRP) residing in hot spring present good opportunity for exploring the limitless possibilities of integral ecosystem processes. Metagenomic analysis further expands the phylogenetic breadth of these extraordinary sulfur metabolizing microorganisms, as well as their complex metabolic networks and syntrophic interactions in environmental biosystems. Through this study, we explored and expanded the microbial genetic repertoire with focus on sulfur cycling genes, biogeochemical cycles, metal resistance genes through metagenomic analysis of mesothermic hot spring Khirganga, located at the Northern Himalayas. The analysis revealed (i) rich diversity of microbial consortia with established roles in S cycling such as *Pseudomonas*, *Thioalkalivibrio*, *Desulfovibrio* and *Desulfobulbaceae* (*Proteobacteria*). Analysis of sequence similarity showed conserved pattern of both *dsrAB* genes (n=178) retrieved from all metagenomes while other sulfur disproportionation proteins were diverged due to different structural and chemical substrates, (ii) the diversity of sulfur oxidizing bacteria (SOB) and sulfate reducing bacteria (SRB) with conserved (r)*dsrAB* suggests for it to be an important adaptation for microbial fitness at this site. Here, the oxidative and reductive *dsr* evolutionary time scale phylogeny, proved that the earliest (not first) *dsrAB* proteins belong to anaerobic *Thiobacillus* with other (*rdsr*) oxidizers, also we confirm that the structural prediction of unassigned DsrAB proteins confirmed their relatedness with species of *Desulfovibrio* and *Archaeoglobus fulgidus* (iii) reconstruction and examination of 41 high and medium qualified metagenome-assembled genomes (MAGs) from at least 12 bacterial and 2 archaeal phyla. Over 1749 genes putatively involved in crucial metabolism of elements *viz.* nitrogen, phosphorous, sulfur and 598 genes encoding enzymes for metals resistance from cadmium, zinc, chromium, arsenic and copper. The MAGs also possess 229 biosynthetic gene clusters dominated by bacteriocins and terpenes could beexploited in medicinal industries, (iv) we employed tailored genome-resolved metagenomics and a novel approach that offers metagenomic overlaps to investigate the core (ECGs) and habitat-specific (HSG) microbial diversity in microbial mat, sediment and water where highest cycling entropy scores suggested abundances of nitrogen-fixing microbes and sulfur utilizing bacteria with iron as essential component for physiological processes like replication, transcription, metabolism and energy generation via respiration.

Contents

17. POOJA KUMARI

Kisspeptin, Dopamine and GnRH Regulate Gonadotropin Release and Oocyte Maturation: In Vivo, in Vitro and in Silico Analyses in the Catfish, Heteropneustes fossilis (Bloch).

Supervisor: Prof. Neeta Sehgal

Th 25667

Abstract

Gonadotropin Releasing Hormone (GnRH) is a key regulator of reproduction which regulates the release of LH from the pituitary cells. GnRH activity is stimulated by many neurotransmitters that in turn resumes meiosis in the oocytes. One of them is kisspeptin which regulates the hypothalamo-pituitary gonadal axis positively, whereas another dopamine acts as the negative regulator. Gene sequences of *kiss2, cdc2* and *cyclin b* from NCBI databank have been translated. Their secondary structure and active protein sites have been identified, to analyze protein-protein interactions. Homology modelling and HADDOCK analysis show interactions between kiss2-GnRH2, kiss2-LHβ and cyclin b-cdc2. Two kisspeptin genes and *kisspeptin receptor* show distribution in several tissues of the catfish. In addition, two dopamine receptor genes (*D2* and *D4*) and tyrosine hydroxylase gene (*Th*) are also expressed. LH surge from pituitary binds to the LH receptors localized in the ovary and stimulates the formation of maturation promoting factor (cyclin b and cdc2 protein complex). Genes (*cyclin b* and *cdc2*) are also expressed which regulate resumption of meiosis in oocytes. Expression of *kiss1*, *kiss2*, *kiss receptor*, *D4 receptor* and *Th* has been corroborated with annual ovarian cycle of the catfish. Transcripts of two genes (*D4 receptor* and *Th*) are upregulated in the brain of catfish during preparatory phase till pre-spawning phase, which indicates that these two genes are responsible for the inhibition of the LH surge via GnRH. Transcripts of *kiss1* and *kiss receptor* are upregulated in the brain during pre-spawning phase whereas, maximum transcripts of *kiss2* have been observed in brain and ovary during spawning phase, suggesting the involvement of kisspeptin for release of LH as surge culminating into maturation and ovulation of oocytes. GnRH analogue induces the release of LH as surge from catfish pituitary leading to meiotic oocyte maturation. In contrast, in the presence of dopamine, GnRH fails to induce meiotic maturation. This inhibitory effect is because of downregulation of *lhb* gene, thereby LH is not released in plasma and finally GVBD in oocytes is not triggered. On the other hand, administration of kisspeptin along with GnRH shows the synergistic effect on expression of *lhb* and release of LH in plasma that leads to maturation and ovulation of oocytes. The interaction between GnRH, dopamine and kisspeptin has been elucidated in the primary culture of pituitary cells. To summarize, the regulatory mechanism of LH release in catfish is under stimulatory control via GnRH and kisspeptin, and inhibitory control by dopamine.

Contents

18. PUKHRAMBAM PUSHPA DEVI

Cloning the Genome of Groundnut but Necrosis Virus (GBNV) and Attempting to Generate Infectious Virus in-vitro from cDNA Clones.

Supervisor: Prof. Rajagopal Raman

Th 25668

Abstract

The obstacles may contribute to the unsuccessful construction of a reverse genetics system for GBNV in the BSRT7/5 cell line. Considering all these factors, there is a need to develop a stable cell line for

thrips or plants that will help overcome the difficulties. In conclusion, we were able to recover the mRNA transcript of each genome segment of GBNV from the cDNA clones but we were not successful in generating the infectious virus. we amplified and constructed full-length cDNA clones of S, M and L genome segments of GBNV from the RNA isolated from GBNV infected cowpea plant by applying long RT-PCR and TA cloning. The strategy is useful in full genome sequencing as well as in the establishment of infectious clones. In addition, this method is cost-effective and can be applied for cloning the whole genome of any other species of tospovirus

Contents

19. RAWAT (Kavita)
Role of Neutrophils in Host-Tumor Interaction and Possible Intervention of Immunomodulatory Rasayana.
Supervisor: Prof. Anju Shrivastava
Th 25657

Abstract

Cancer initially grows as a local disease but eventually turns into a complex systemic illness that gradually affects the entire body of the tumor bearing host. The systemic effects which include metastasis, inflammation, thrombosis and cachexia, leads to functional impairment of various organs with disease progression. These systemic effects are a significant contributor to many cancer-related deaths. Importantly, majority of these systemic manifestations appear to arise from chronic inflammation caused by the growing cancer cells and aberrant immune response of the host. Various reports suggest that these states of immune dysregulation and excessive inflammation target different organ systems with potentially lethal or highly morbid conditions. Therefore, the view of cancer as a systemic disease has emerged as a research spotlight wherein the quest is to comprehend the underlying mechanisms impacting transformation of a localized disease to a complex systemic ailment. Neutrophils, which are most important effector cells of innate immunity, are being realized to play a crucial role in tumor biology. Due to their supposedly involvement in tumor progression, neutrophils have recently become the subject of intense research with focus on association between inflammation and cancer progression. Of note, cancer patients show remarkable increase in peripheral blood neutrophil count and their infiltration in tumors. Based on this observation, neutrophil-to-lymphocyte ratio (NLR), an indicator of inflammation, has been adopted as a prognostic sign of poor survival in cancer patients. So far, substantial reports have solely focused on the role of neutrophils particularly within the tumor microenvironment or at the inflammation site; however, whether they impact systemic milieu or not is still a question. As cancer is a systemic disease, therefore, it is worthwhile to explore whether and how neutrophils contribute to systemic deterioration in cancer and if yes, can it be reinstated by potent immunomodulators. A number of chemically synthesized compounds and monoclonal antibodies are currently being used as immunomodulatory agents. However, due to the occurrence of adverse effects of the synthetic compounds, natural immunomodulators are considered to be the potential agents to be used in therapeutic regimens. Among the vast library of medicinal plants, *Tinospora cordifolia* is well known for its valuable phytoconstituents with several therapeutic efficacies. In the Ayurvedic literature, *Tinospora cordifolia* is known for its huge applications in the treatment of various diseases such as jaundice, rheumatoid arthritis, urinary disorders, skin diseases, diabetes, anemia, inflammation, and allergic conditions. Traditional studies suggest its strong immunomodulatory role and experimentally very few reports are available that too limited to its action on macrophages. However, its effect on neutrophils, which are the most dominant immune cells and crucial players in chronic inflammatory diseases, has not been deciphered so far. Based on the available

studies, we were interested to explore whether and how neutrophils contribute to systemic deterioration in cancer and if yes, can it be reinstated by potent immunomodulatory rasayana like *Tinospora cordifolia*? To answer these questions, we utilized Dalton's Lymphoma (DL) tumor model which is a spontaneous T-cell lymphoma which originated in thymus of Balb/c mice. It is well characterized, reproducible and is considered as a good model system for *in vivo* studies. Following objectives were framed to answer the question: To assess neutrophil count in blood and their infiltration in vital organs of tumor bearing host with tumor progression. To examine the systemic impact of neutrophils in tumor-bearing host. To investigate the role of *Tinospora cordifolia* in taming neutrophil function to ameliorate cancer-induced systemic deterioration. To explore the involvement of neutrophils in mediating systemic effect with tumor progression, we first evaluated their presence in peripheral blood and vital organs of DL-bearing mice at different time points with tumor progression. We characterized neutrophils using anti-Ly6G which is a neutrophil marker and performed immunofluorescence, flow cytometry and immunohistochemistry to determine their presence. Neutrophils are inflammatory cells and their aberrant accumulation is often associated with tissue injury. Therefore, to examine organ function, we first investigated the histoarchitecture of vital organs and then evaluated the biochemical enzyme levels at different time points of tumor growth. We were further interested to assess the neutrophil function and therefore, examined the expression of neutrophil-derived granular cargoes such as neutrophil elastase (NE), myeloperoxidase (MPO), MMP-8, MMP-9 and cathepsin G in the vital organs of tumor-bearing host. Next, we evaluated the immunomodulatory potential of *Tinospora cordifolia* wherein the extract treatment was scheduled at early, mid and advanced stages of tumor growth at a dose of 400 mg/kg b.wt. for 30 consecutive days. In the present study with Dalton's lymphoma tumor model, we observed gradual increase in polymorphs and total leukocyte count with parallel reduction in lymphocyte number at different time points of tumor growth conforming to the established cancer norms. We confirmed the presence of neutrophils using anti-Ly6G and found a significant increase in Ly6G+ cells in the peripheral blood. Surprisingly, we also found a systemic presence of neutrophils as the number of Ly6G+ cells were markedly high in all the examined organ of tumor-bearing mice as compared to the control. Histology images also showed cellular infiltrations and disturbed tissue architecture that aggravated with tumor progression. Simultaneously, there was a significant organ dysfunction observed as assessed by alteration in biochemical enzyme levels. Another interesting observation of the study was that the infiltrating neutrophils displayed hyperactivation as seen by release of toxic granular cargoes including NE, MPO, MMP-8, MMP-9 and cathepsin G which may be responsible for the observed gradual concomitant tissue damage with tumor progression. In the next part of the present study, we aimed to evaluate the possible immunomodulator to reinstate the neutrophil number and activation as an approach to ameliorate the systemic damage with cancer progression. For this, we evaluated TCE extract which was administered at early, mid and late stage of tumor growth. We observed that TCE administration at early and mid-stage of tumor significantly down-regulates neutrophil count in peripheral blood and their infiltration in vital organs of tumor-bearing mice. It also ameliorated hyperactivation by suppressing the heightened release of toxic granular cargoes. Further, TCE treatment maintained histoarchitecture and biochemical enzyme levels at different stages of tumor growth. In addition, it restored the body weight, girth size and morphology of lymphoid organs, with a remarkable increase in survival of the tumor-bearing mice at different stages of tumor growth. To our knowledge, this is the first *in vivo* report which demonstrates the important role of neutrophils in mediating systemic damage and its regulation by potent immunomodulatory rasayana *Tinospora cordifolia*. We convincingly showed that: High neutrophil infiltration in peripheral blood and vital organs was accompanied with the excessive release of its key effector molecules (NE, MPO, MMP-8, MMP-9 and Cathepsin G). The excessive release of effector molecules represents the hyperactive response of neutrophils, which might be leading to the tissue damage and subsequent organ dysfunction during tumor progression. TCE administration at early and mid-stage of tumor growth regulates neutrophil infiltration and hyperactivation which ameliorates cancer-induced systemic damage and further substantiates the anti-tumor actions of TCE. We conclude that taming neutrophils could be a potential therapeutic approach to prevent systemic deterioration in cancer patients. Further, detailed mechanistic studies will present a comprehensive outlook for the applicability of TCE as a potent immunomodulatory drug which can ameliorate systemic damage and improve quality of life in cancer patients.

Contents

1. Introduction 2. To assess neutrophil count in blood and their infiltration in vital organs of tumor bearing host with tumor progression 3. To examine the systemic impact of neutrophils in tumor-bearing host 4. To investigate the role of Tinospora cordifolia in taming neutrophil function to ameliorate cancer-induced systemic deterioration. Summary. Publication and conferences.

20. SENGUPTA (Madhumita)
Radiobiological Investigations on Reproductive Competence of Radio-Sterilized Female Moth, Spodoptera Litura (Fabr.) for its Combined Release with Sub-Sterilized Males in Employment of Radiation Mediated 'Inherited Sterility Technique' for Pest Suppression.
Supervisor: Prof. Rakesh Kumar Seth
Th 26523

Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Determination of a suitable radiation dose to be administered on female moths by studying their dose response in terms of calling behaviour, mating behaviour and reproductive success of S.litura 5. Effect of radiation on the profile of pheromones of female moths in correlation with expression of genes associated with reproduction 6. Effect of radiation on the pheromone perception of male S. litura in terms of orientation behaviour and odorant receptor gene SlituOR expression in 130 Gy treated male moths and their F1 male progeny 7. Effect of scale dislodgement on the mating efficiency and competitiveness of irradiated moths for combined release 8. Summary, conclusion and future perspectives. References.

21. SHAILENDRA KUMAR
Influence of Farnesol on Growth, Development and Reproduction of Red Cotton Bug Dysdercus Koenigii Fabricius (Heteroptera: Pyrrhocoridae).
Supervisors: Prof. Kamal Kumar Gupta and Prof. Rajiv Aggarwal
Th 25675

Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Influence of farnesol on growth and development of dysdercus koenigii 5. Influence of farnesol on reproductive bioactivities of dysdercus keonigii 6. Effects of farnesol on haemocyte profile of fifth instar nymphs of dysdercus koenigii 7. Effect of farnesol on proteomics of haemolymph of dysdercus koenigii 8. Influence of farnesol on juvenile hormone titre in dysdercus koenigii. Summary. References. Publications and paper presentations.

22. SHARMA (Vishal)
Molecular and Metabolite Profiling of Tuberculosis Patients Using Stool Samples.
Supervisors: Prof. Rina Chakrabarti and Prof. Yogendra Singh
Th 25655

Abstract

Tuberculosis (TB), an airborne infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), remains a major killer globally. *Mtb* primarily infects the lungs, causing pulmonary tuberculosis (PTB), but also

manifests in other body parts as extrapulmonary tuberculosis (EPTB). Several studies have reported that a large population has latent TB, which has the potential to progress to active TB. In addition, host immunity, age, gender, co-occurrence of other diseases, and, most recently, the gut microbiome have been demonstrated to impact the susceptibility to TB and its pathogenesis, either directly or indirectly. Early and accurate diagnosis and proper treatment are the backbones of disease eradication. The advancements in TB molecular diagnostics such as Xpert MTB/RIF and Xpert MTB/RIF Ultra strengthen early TB diagnosis, yet the sample type for diagnosis of EPTB, pediatric TB and smear-negative TB cases are still lacking. Therefore, this study demonstrates the potential of non-invasive stool samples using Xpert MTB/RIF Ultra for the diagnosis of smear-negative PTB and EPTB cases. Furthermore, this study also compares the diagnostic accuracy of Xpert MTB/RIF on stool samples and other available sample types using meta-analysis. In addition, another aspect of the study shows the differences in gut metabolites during TB infection using 1-dimentional 1H-NMR spectroscopy and GCMS. The results showed the upregulation of valine, serine and taurine and downregulation of methionine and suberic acid in the gut of TB patients in comparison to healthy individuals. Since, TB is an inflammatory disease; dysregulation of metabolites can play a crucial role in the regulation of host oxidative stress and inflammation. The potential gut metabolite biomarkers identified in this study have the ability to significantly contribute to the development of early diagnostics, personalized therapies and treatment for the restoration of metabolites alteration and subsequent relief.

Contents

23. SHIVANI KUMARI
Potential Application of Isolated Jeotgalicoccus sp. CR2 for the Degradation of Chloramphenicol and Remediation of Multiple Heavy Metals.
Supervisor: Prof. D. K. Singh
Th 26055

Abstract

Chloramphenicol and heavy metals are continuously polluting our water bodies from various sources and show a wide range of toxicity to aquatic life and humans. The conventional techniques used for their remediation are not effective in the present scenario. Bioremediation is the most efficient and cost-effective technique for the antibiotic and heavy metal removal from the water bodies. Recently, various bacteria, fungi and algae have been isolated and checked for the bioremediation of Chloramphenicol and heavy metals for the aquatic ecosystems. Moreover, many emerging technologies including sponges, microalgae and biochar are being tested for the remediation of various antibiotics and heavy metals in eco-friendly manner. Based on the above literature survey, the objectives of the thesis have been designed for the isolation of chloramphenicol and heavy metal resistant bacteria. Further, the metal removal efficiency and antibiotic degradation efficiency has been checked for the isolated bacterial strain. The high degree of urbanization and industrial revolution led to heavy metal pollution and accumulation in the water bodies. These heavy metals are highly toxic for aquatic life and enter the food chain through the seafood and irrigation practices in the crop fields. In addition to the heavy metals, antibiotic pollution is also one of the major concerns in Yamuna River water. Nowadays, antibiotics consumption has increased many times which is having adverse effect on environment and contributing in bacterial resistance finally leading to multi-drug resistance. This study focused on the isolation and investigation of

multi-metal resistant and Chloramphenicol resistant bacterial strain from the Yamuna River water samples. The isolated *Jeotgalicoccus* sp. CR2 strain was able to tolerate multi-metal (Zn2+, Pb2+, Cu2+, and Ni2+) stress up to 20 ppm. Moreover, Chloramphenicol resistance was also studied for the strain and it was found chloramphenicol tolerant up to 20 mg/L. In further objectives, metal removal efficiency and chloramphenicol degradation potential has been explored The morphological characterization of the isolated strain in heavy metal stress was performed using SEM and FTIR spectroscopy analysis. SEM micrographs revealed that the metal-laden cell surface deteriorated and the cell shape was distorted as compared to the control cell. FTIR spectral analysis depicted that carboxyl, amide, and phosphate groups are the main functional groups associated with the metal ion interaction and its biosorption on the bacterial cell. The metal removal efficiency of the *Jeotgalicoccus* sp. CR2 strain detected up to 96.65 % using AAS analysis. Further, through LCMS protein analysis, three unique protein bands corresponding to three enzymes were identified, which are responsible for heavy metal removal from the bacterial cell. And based on the function of these enzymes, proposed pathway of heavy metal removal is presented. This potential multi-metal resistant bacterium can be further studied at the gene level and can be implemented in a bioreactor for the bioremediation of the multiple heavy metals from the aquatic ecosystems The morphological characterization of the isolated strain in the Chloramphenicol stress with the biotic control was performed using SEM and FTIR spectroscopy analysis. SEM micrographs revealed that the antibiotic-laden cell surface deteriorated and the cell shape was distorted as compared to the control cell. Cells were forming clusters due to the production of EPS by the bacterial cell in response to the Chloramphenicol degradation by the bacteria. The FTIR spectroscopy results suggested that carboxyl and amine groups are the main functional groups associated with the Chloramphenicol interaction with the *Jeotgalicoccus* sp. CR2 strain. The effective Chloramphenicol percent degradation of the *Jeotgalicoccus* sp. CR2 strain was estimated as 89.12% by using UPLC chromatogram. This potential Chloramphenicol resistant bacteria can be further studied at the gene level and can be implemented in a bioreactor for the bioremediation of the antibiotics from the aquatic ecosystems.

Contents

24. SHRIVASTAVA (Nidhi Krishna)
Study of Immune Function in Drosophila Melanogaster Selected for Divergent Life History Traits.
Supervisor: Prof. Mallikarjun Shakarad
Th 25913

Abstract

The aim of the study was to address whether selection for development time and age of reproduction will alter the immune function. I used three selection lines of *Drosophila melanogaster* in this study. First were lines selected for faster development and late reproduction (FLJ), second were lines selected for faster development and early reproduction (FEJ) and third were their common ancestral control lines (JB) Comparative whole genome data analysis of FLJ and JB populations showed high impact variants in two immune function genes- *Tep3* and *NimB5* of which transcript level of *Tep3* was significantly high. Further, percent phagocytosis and crystal cell number were significantly higher in FLJ's compared

to JB's. On assaying the immune function in *Drosophila* selected for faster development and early reproduction (FEJ), there was upregulation of transcript level of genes associated with production of prophenoloxidase enzyme namely *PPO1* and *PPO2* and antimicrobial peptides- *Drosomycin, Defensin, Diptericin, Cecropin* and *Drosocin* in the FEJ populations along with phagocytic receptors *NimC1* and *eater.* Transcript level of *eiger,* a cytokine which plays a role in activation of immune response under nutritional deprivation was not different. Transcript level of *edin* a gene reported to get upregulated under infection was significantly higher in FEJ populations. Further, the transcript level of genes involved redox homeostasis- those responsible for ROS production (*Duox* and *Nox*) and ROS scavenging (*SOD* and *Catalase*) were also significantly higher in FEJ populations.

Contents

25. SINGH (Akansha)
Investigation of Molecular Basis of Lipid Metabolism in *Drosophila* Model of Huntington's Disease.
Supervisor: Prof. Namita Agrawal
Th 25658

Abstract

Huntington's disease is a highly incapacitating genetic disease, with no known cure existing till date. Extensive structural and functional remodelling of the brains of diseased individuals evoke many of the hallmark symptoms of HD. However, it is becoming clearer that patients suffer from multiple peripheral comorbidities which may or may not be secondary to the disintegrating neurobiology. The metabolic components of the disease represented by energy deficit, oxidative stress, unexplained weight loss, increased insulin resistance and deficiency of key metabolic substrates have been increasingly recognised as critical determinants of disease outcome. Accordingly, delineating the trajectories of central and peripheral metabolic alterations and finding a link between them may prove to be essential in understanding the nature of this multifaceted disease. Brain metabolic defects were amongst the earliest dysfunctionalities identified in the disease pathophysiology. Sooner, the idea of HD being a multiple organ disease embarked. Key biomolecules identified over the years to be altered in HD include glucose, insulin, cholesterol, leptin, ghrelin, branched chain amino acids and substrates of TCA, ETC and glycolysis. While hypothalamus is the master regulator of the metabolic homeostasis, hypothalamic-endocrine aspects do not explain the whole picture of metabolic alterations occurring in HD on its own, as many anomalies have been reported to occur independent of it. A metabolic component has also been well professed in other neurodegenerative diseases such as Alzheimer's and Parkinson's. HD offers an advantage of being caused by a single gene mutation which can be readily identified by genetic screening. Moreover, the disease expression can be predicted by CAG repeat length and the patients and families can be made aware of the treatment options well before hand. Clinical trials of many candidate therapeutic compounds have been carried out at different phases. IONIS-HTTRX, an antisense oligonucleotide targeting Huntingtin pre-mRNA has successfully been able to reduce the concentrations of mHTT protein from CSF and was the only disease-modifying

candidate. However, unfortunately, Phase III trial of IONISHTTRX was stopped recently apparently due to inefficacy of the treatment. Anaplerotic therapy and therapies targeting mitochondrial dysfunction have gained momentum against many neurodegenerative diseases. It would be only reasonable to expect the strategies with disease-modifying potential working best with combinatorial treatments restoring metabolic homeostasis. It is most plausible that profound metabolic dysfunction existing with HD might be a driving force for the disease progression and severity. As mHTT protein is expressed ubiquitously and is able to interact with several transcription factors, vesicular transport proteins and signalling proteins, it would be interesting to know whether the organ-specific defects are due to the cell-autonomous effects of mHTT or independent of it. Many confounding results exist among clinical and experimental studies, which needs to be validated with large cohort, multicentre and longitudinal studies. Further mechanistic insights to metabolic alterations co-existing with HD will help us comprehend nature of the disease, develop novel biomarkers and devise new treatment options.

Contents

26. SINGH (Anoop)
Understanding the Evolutionary Success of Mycobacterium Tuberculosis by its Genetic Signatures and Host-Specific Microbial Markers.
Supervisors: Prof. Yogendra Singh and Dr. Asani Bhaduri
Th 25665

Abstract

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), remains the primary cause of human deaths by a bacterial infection. It has evolved as a successful human pathogen from a pool of environmental bacteria, and in this process, its genome has undergone a series of changes caused by horizontal gene transfer (HGT), gene duplications, genomic deletions, and vertical genome reduction. These genetic signatures are ideal candidates for understanding potential patho-adaptation acquired by *M. tuberculosis* to survive inside the host environment successfully. Moreover, a pathogen may encounter competition from the commensal microbiota inside the host environment. As a result, *M. tuberculosis* may have devised some strategies during its evolution to overcome the resistance from the commensal microbiota. Therefore, any dysbiosis in the host-microbiome can affect host-susceptibility to TB as it plays a crucial role in host immune regulation. This study demonstrates the importance of *M. tuberculosis* complex (MTBC) specific genetic markers in patho-adaptation followed by a comprehensive analysis of one of the clinically relevant markers, the CRISPR-Cas system, to identify the genetic signatures of its evolution and possible links with pathogenesis in the MTBC members. This study also provides evidence of IS6110-derived variation and HGT-mediated origin of the CRISPR-Cas locus in the MTBC, which can help us to better understand the strain-specific variations in *M. tuberculosis* lineages. Furthermore, another aspect of the study shows the host-microbiome dynamics during TB infection. We found gut microbial markers associated with dysbiosis in TB patients. Our results showed under-representation of short-chain fatty acid (SCFA) producers in the gut microbiome of TB patients, indicating impairment of the immune function, as SCFA producers are known to play a crucial role in the immuno-regulation. The pathogen-associated genetic and host gut microbial markers described in the study are clinically relevant and have diagnostic and therapeutic implications.

Contents

27. SINGH (Chandra Kant)
Ascertaining the Gut Bacterial Diversity of Radio-Sterilized Spodoptera Litura (Fabr.) and its F1 Progeny: Correlation of Gut Bacterial Assortment with Viability of Moths used in 'Inherited Sterility Technique' for Pest Suppression.
Supervisor: Prof. Rakesh Kumar Seth
Th 26525

Contents

28. SODHI (Kushneet Kaur)
Potential Application of Isolated Alcaligenes sp. MMA in the Amoxicillin Degradation and Remediation of Heavy Metals, and Synthesis of Amoxicillin-Iron (III) Complex for the Enhanced Antibacterial Activity.
Supervisor: Prof. Dileep Kumar Singh
Th 25674

Abstract

The river Yamuna is harnessed by pollutants including the likes of antibiotics and heavy metals. The use of microorganisms for the remediation of these pollutants is a lucrative option. In view of this, the present thesis work consisted of five objectives. In the first objective, antibiotics and metals were detected in the Yamuna. Amoxicillin was the most abundant antibiotic which was detected in the Yamuna. Multiple heavy metals were also detected in the Yamuna. Along with the detection of pollutants, the structural and functional profile of the bacterial community in the Yamuna were also assessed. The phylum Proteobacteria was the most abundant phylum followed by the phylum Bacteroidetes. The second objective begins with the isolation and characterization of the amoxicillin degrading bacteria, and amoxicillin-induced alteration in the protein profile was also studied. Bacteria were able to degrade amoxicillin and the metabolites were identified using mass spectrometry (LC-MS/MS) analysis. DNA-dependent RNA polymerase and porins were expressed in amoxicillin stress in *Stenotrophomonas* sp. WA5 and *Alcaligenes* sp. MMA. Further, in the third objective, the maximum tolerance index of the amoxicillin-resistant bacterial strains was studied, and *Alcaligenes* sp. MMA was selected for metal removal. The bioaccumulation of the heavy metals (Cu2+, Cd2+, Cr6+, Ni2+, Zn2 +) was observed in the range of (44-88%). In the fourth objective, the *Alcaligenes* sp. MMA bioremediation capabilities were explored. Various heavy metals and antibiotic-resistant genes were present which further proves the bioremediation potential of the bacteria. In the fifth objective, we successfully synthesized the Amoxicillin-Fe (III) complex in-vitro and studied its antibacterial activity. The metal complex has enhanced antibacterial activity against amoxicillin-resistant bacterial strains. In

conclusion, bacteria and their enzymes have the potential for bioremediation. Moreover, the antibiotic metal complex can be used as a novel compound that can have enhanced activity over many different antibiotics.

Contents

1. Introduction 2. Review of literature 3. Detection of contaminants (antibiotics and metals), and assessing the structural and functional profile of the bacterial community in the river Yamuna 4. Isolation and characterization of amoxicillin degrading bacteria and studying the amoxicillin-induced alteration in its protein profile 5. Assessing the multiple metal tolerance and removal by the amoxicillin resistant bacterial strains 6. Exploration of bioremediation capabilities of alcaligenes sp. strain MMA 7. Synthesis and characterization of amoxicillin-iron (III) complex, and assessing the antibacterial activity 8. Summary. List of publications.

29. TYAGI (Ekta)
Characterization of Rv1985c of Mycobacterium tuberculosis as Nucleoid Associated Protein.
Supervisors: Prof. Rakesh Kumar Seth, Prof. Yogendra Singh and Prof. Ranjana Seth
Th 26524

Contents

1. Review of literature 2. To study phylogenetic analysis and interactomics study unveils gene co-optive evolution of LysR-type transcription regulators across non-pathogenic, opportunistic and pathogenic mycobacteria 3. To identify and characterize LTTRs from Mtb as nucleoid associate protein. References. Appendix. Publications and conferences.

30. YADAV (Karuna)
Identification and Characterization of Putative Interacting Protein Partners of Dengue Virus Envelope € Protein in the Insect Vector *Aedes Aegypti*.
Supervisor: Prof. Rajagopal Raman
Th 25669

Abstract

Dengue fever (DF) is an arboviral infection, transmitted to humans by the mosquito vector *Aedes aegypti*. Dengue virus (DENV) of the genus Flavivirus is the causative agent of DF. The DENV has four separate antigenic groups also known as DENV serotypes. The average number of DF cases is approximately 400 million across the globe. The infection from one serotype can provide lifelong immunity against the same serotype but cross-immunity against other serotypes is either temporary or partial. In the case of humans, secondary infection by other serotypes increases the chances of developing severe forms of dengue-like dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). In the past few decades, dengue has emerged as a major public health concern, especially in developing nations. The infection is endemic in different countries of Africa, America, and South-East Asia. Asia contributes a maximum (~70%) to the global burden of DF infections. At present, there is no vaccine available that is effective against all four DENV serotypes. The DENV envelope (E) protein is primarily involved in the viral attachment to host cells. Further, it is involved in facilitating the viral entry into the host cells using membrane fusion or cell-mediated endocytosis. A better understanding of how DENV interacts with possible receptors present on the host cell surface is necessary and identification of such receptors can be very useful in reducing DENV transmission. The previous studies on the interaction of DENV with *Ae. aegypti* at the cellular level are limited. The current study was primarily focused on understanding interactions of DENV with *Ae. aegypti* with two objectives. In the first objective mucin protein of *Ae. aegypti* was identified as a potential candidate receptor of DENV-EDIII using phage display library screening. The dot blot assays and *in-vitro* pull-down assay

result suggested the positive interaction of EDIII (envelope domainIII) with mucin protein. The expression of mucin protein was localized in the *Aedes* midgut, as well as co-localization of mucin protein with DENV in the DENV infected midgut. Antibody inhibition assay of the mucin protein results in a decrease in the viral titer in adult *Ae. aegypti*. Molecular docking between E and mucin protein suggested the possible amino acids residues involve in the interaction between the two proteins. The second objective involves the construction of *Ae. aegypti* gut cDNA library in yeast and the employment of Yeast two-hybrid assay to identify the candidate interacting proteins with DENV-EDIII. Polyubiquitin protein was identified as a interacting partner and further biochemical assays were performed to understand the interaction. The dot blot and *in-vitro* pull down assay suggested the interaction between polyubiquitin protein and EDIII.

Contents

31. YADAV (Priya)
Characterization and Dynamics of the Bio-Molecules Responsible for Sperm Activation in a Serious Noctuid Pest, Spodoptera Litura (Fabricius) vis-à-vis Ionizing Radiation: Potential for Pest Suppression.
Supervisor: Prof. Rakesh Kumar Seth
Th 25666

Contents