CHAPTER 65

ZOOLOGY

Doctoral Theses

669. BAJAJ (Divya)
Heme Oxygenases in theDeveloping Wild Type and Mutant Hmox1 Mouse Embryos.
Supervisor : Prof. Sharmila Basu-Modak
Th 22444

Contents

1. Introduction. 2. Materials and methods. 3. Developmental profile of hmox proteins during embryogenesis of wild type hmox 1 mice. 4. Immunohistochemical analysis of the hmox proteins in wild type embryos. 5. Analysis of the effect of hmox 1 mutation on organ development in late gestation embryos. 6. Investigation of discrepancies observed in prenatal lethality of the hmox 1 mutant embryos and mice. General discussion and bibliography.

670. DATTA (Debika)
Studying the Pathogenesis of Mycobacterium Fortuitum in Piscine Model.
Supervisor : Dr. Shibnath Mazumder
Th 22130

Abstract

Mycobacterium fortuitum, rapidly growing atypical mycobacteria is the prime cause of mycobacteriosis in a wide range of hosts, including human and fish. We have developed piscine-M. fortuitum infection model using Clarias sp. (Catfish) and Danio rerio (Zebrafish). The infection with 1×10^7 CFU of M. fortuitum induced chronic infection characterised by non-necrotic granuloma in catfish. A reverse relation between TNF-α and IFN-γ production with bacterial burden were noted. Memory response induced by M. fortuitum conferred protection against lethal challenge. Zebrafish were infected with 1×10^6 CFU of M. fortuitum and it was observed that the Th1 and pro-inflammatory response significantly high at early stage of infection while Th2 and anti-inflammatory response was prominent at later stage. TLR-2 expression was noted at very early stage of infection. We hypothesize that TLR-2 mediated Th1 response induces up-regulation of pro-inflammatory cytokine production that conferred protection to the host by eradicating intracellular M. fortuitum. In vitro study revealed that infection of headkidney macrophages from Clarias with M. fortuitum (MOI 1:10) leads to caspase dependent apoptosis. M. fortuitum triggers intracellular-calcium (Ca^{+2}) influx leading to the activation of calmodulin (CaM), protein kinase C alpha (PKCα) and Calmodulin kinase II gamma (CaMKIIg). We noted that CaMKIIg activation is regulated by CaM as well as PKCα-dependent superoxide anions which in turn activate ERK1/2. Crosstalk between ERK1/2 and NOS2 shifts the balance in favour of extrinsic pathway of

apoptosis. We also report that M. fortuitum induces Ca^{+2}-mediated ER-stress characterized by the over expression of CHOP and BiP. Augmented ER-stress promoted calpain mediated caspase-12/caspase-9 activation. Ca^{+2} imbalances led to the alteration in mitochondrial membrane permeabilization, cytosolic release of cytochrome c eventually activating intrinsic pathway of apoptosis. We summarize that M. fortuitum induces caspase-dependent apoptosis of fish macrophages where intracellular Ca^{+2} mobilizations plays crucial role to trigger the downstream signalling cascade.

Contents

671. DIMRI (Manali)
Screening and Identification of Hematopoietic Stem Cell Expanding Small Molecule Clinical Compounds as Radiation Countermeasure Agents.
Supervisors : Prof. Rina Chakrabarti and Dr. I. Prem Kumar
Th 22132

Contents

672. G ANJALI
Molecular Mechanisms Involved in FSH Mediated Glucose Metabolism in Granulosa Cells.
Supervisor : Dr. Rita Singh
Th 22133

Contents

673. HELIANTHOUS
Comparative Genomics and Taxonomical Characterization of Sphingomonads
Supervisor : Prof. Rup Lal
Th 22438

Abstract

The family Sphingomonadaceae consists of five genera: Sphingomonas, Sphingobium, Novosphingobium, Sphingopyxis and Sphingosinicella commonly called as

sphingomonads. They are of particular interest due to hexachlorocyclohexane (HCH) degradation potential corresponding to the presence of lin genes. Research work carried out in the thesis has been described in four chapters. The first chapter includes draft genome of Sphingobium baderi LL03^T, isolated from HCH-dumpsite, Czech Republic. Data obtained from Illumina and 454 GS-FLX titanium was assembled into 92 contigs using ABySS1.3.3 assembler. The genome analysis revealed its coding density (88.96%), GC content (63.5%) and genome size (~4.85Mb). Also, presence of a well maintained CRISPR element and intact phage, both belonging to Burkholderia genus suggests evidence of phage-host interaction. Second chapter includes comparative genomic analysis of nine Sphingobium strains which revealed high conservation in sequence identity and copy number variation in upper and lower lin pathway while linKLMN operon was most diverged. The genome analysis also reinforced role of IS6100 and plasmids in lin genes acquisition with an inter-genus plasmid pool between Sphingobium and Sphingomonas. Chapter third involves the use of Single Molecule Real Time Sequencing (SMRT) technique to complete the genome of a potent HCH degrader, Sphingobium indicum B90A. The draft genome of strain B90A was announced earlier which was re-sequenced and assembled into one chromosome (3,654,322bp) and three plasmids, pSRL1 (139,218bp), pSRL2 (108,430bp) and pSRL3 (43,761bp). lin genes were identified as linA2, linB, linF, linGHIJ and linKLMN were located on chromosome, linA1, linC on pSRL1 and linDER on pSRL3. Fourth chapter deals with taxonomic characterization of a novel strain isolated from HCH-dumpsite, India using polyphasic approach. Genotypic, phenotypic and chemotaxonomical characteristics of strain R11H^T classified it as Sphingopyxis flava R11H^T. This study can now add to the on-going efforts of developing bioremediation techniques by using consortium of better HCH degrading bacterial strains

Contents

674. KAYESTH (Sunil)
Influence of Plant Extracts on Survival, Longevity, Growth, Development and Reproductive Bioactivities of Dysdercus Koenigii
Supervisor : Dr. Kamal Kumar Gupta
Th 22440

Contents

extracts of catharanthus roseus, ocimum santum and lantana camara. Summary and References. Publications and paper presentations.

675. KHANGEMBAM BRONSON KUMAR
Digestive Enzyme Profile of Carps: Hormonal Manipulation, Biochemical Properties and in Vitro Digestibility Study.
Supervisor : Prof. Rina Chakrabarti
Th 22131

Contents

1. General introduction 2. Review of literature 3. Effect of exogenous bath treatment of cortisol and triiodothyronine on the growth and digestive enzyme activities of catla Catla catla during ontogenic development 4. Estimation of protein content and digestibility of some plant proteins in carps rohu labeo rohita and common carp cyprinus carpio using in vitro pH stat method 5. Isolation, purification, characterisation and potential applications of serine protease trypsin from the digestive system of the three Indian major carps catla Catla catla, rohu labeo rohita and mrigal cirrhinus mrigala 6. Summary and conclusions 7. References 8. Publications.

676. KHUSHBOO
Evolution of Critical Size and its Role in LIfe History Traits of Drosophila Melanogaster Populations Simultaneously Selected for Two Traits.
Supervisor : Prof. Mallikarjun Shakarad
Th 22445

Contents

1. Introduction. 2. Materials and methods. 3. Evaluation of minimum critical size under simultaneous selection for faster development and extended longevity. 4. Implications of simultaneous selection for faster development and extended longevity on energy homeostasis. 5. Effect of critical size on survival and reproduction. References and list of publications.

677. MAJUMDAR (Gaurav)
Molecular Basis of Photoperiodic Induction of Seasonal Response in Migratory Redheaded Bunting, Emberiza Bruniceps.
Supervisor : Prof. Vinod Kumar
Th 22129

Abstract

The research included in this thesis focused on the mechanism(s) of photoperiod-induced effects on seasonal processes in a long-distance songbird migrant, the redheaded bunting (Emberiza bruniceps). The thesis has been organized into 4 chapters, each dealing with a specific question. Although we measured the expression of genes implicated in the photoperiodic perception (rhodopsin, neuropsion, melanopsin, peropsin) and induction (eya3, tsh-beta, dio2, dio3) in most study, a few experiments also looked at expression levels of metabolic genes, and at immunohistochemical expression of eya 3 and tsh-beta. The chapter I concludes that rapid switching of hypothalamic gene expression underlies photoperiod-induced seasonal plasticity and regulates transitions from photosensitive to photostimulated

and from photorefractory to photosensitive states in migratory songbirds. We further showed that entrainment of circadian rhythm of photoinducibility determined genetic regulation of the species-specific critical daylength in chapter II. Using modified first-day release protocol, in chapter III we showed localization of photoperiodically induced peptides in the redheaded bunting hypothalamus which were heither to been demonstrated at the transcriptional levels. Finally chapter IV study showed the expression of genes implication in photoperiodic perception and induction in the bunting retinae. Overall, our results support the idea that a photoperiodic species consistently responds at gene levels to changes in the annual photoperiodic cycle. We conclude that there is molecular basis of transisitons in photoperiod-induced phenologies with seasons in redheaded buntings.

Contents

678. MISHRA (Monika)
Evaluation of Bio-Efficacy of Thevetia Neriifolia Extracts on the Growth dnd Development of helicoverpa armigera (Lepidoptera: Noctuidae).
Supervisor : Dr. Sarita kumar and Dr. Kamal Kumar Gupta
Th 22453

Abstract

Helicoverpa armigera, cotton boll worm, is a common agricultural pest causing heavy crop losses worldwide. Current study demonstrates the impact of hexane and methanol extracts of leaves and stems of yellow oleander Thevetia neriifolia, on the growth and development of bollworm. The extracts were incorporated in larval diets and screened for their larvicidal potential and feeding deterrence. Selected extracts were assessed for their larval growth-inhibitory and growth-regulatory effects; and the reproductive fitness of emerged adults. Impact of T. neriifolia extracts was also explored on the larval ingestive toxicity causing physiological and biochemical changes in the larval midgut. Various functional groups and components present in the extracts were identified using FTIR and GC-MS techniques. The results showed that none of the extracts could cause significant larval mortality; though hexane leaves extract proved to be effectual feeding deterrent. The stem extracts delayed larval development, decreased larval and pupal weights, and significantly reduced per cent adult emergence, oviposition and fertility of H. armigera. Further, stem extracts did not affect larval diet consumption and digestibility, but affected their metabolic efficiency in food digestion and assimilation. The extract-fed larvae also showed significantly reduced transaminase and phosphatase enzyme activities in the midgut, the maximum reduction observed in T. neriifolia stem methanol extract-fed larvae. A positive correlation was observed between the extract concentration in the diet and the extent of midgut damage, resulting in more severely damaged epithelial lining, distorted epithelial cells, increase in the gut lumen and exfoliation of epithelium from the

basement membrane. The FTIR and GC-MS analysis showed presence of various bioactive phyto-components in the T. neriifolia extracts probably responsible for their variable bio-efficacy against H. armigera. Present study attempts to provide a non-detrimental approach which poses less threat to the mankind and can help in management of H. armigera population in the fields.

Contents

1. Review of Literature 2. Matrials and Methods 3. Screening of thevetia neriifolia exracts for cidal and antifeedant potential against helicoverpa armigera 4. Impact of thevetia neriifolia stem extracts on the Growth and Development of helicoverpa armigera 5. Impact of thevetia neriifolia stem extracts on the nutrition, gut enzymes and gut histological architecture of helicoverpa armigera 6. Identification of phyto-components in the thevetia neriifolia extracts using FTIR and GC-MS spectral analysis 7. Discussion. Summary and conclusions. references. Annexure-research publications.

679. MUKHERJEE (Udita)
Genetic Manipulations and Genomic Analysis of Rifamycin Producer, Amycolatopsis mediterranei.
Supervisor : Prof. Rup Lal
Th 22139

Abstract

The members of the genus Amycolatopsis are prolific rifamycin producers and thus it was chosen as the suitable organism to base this study upon. Even though the rifamycin polyketide synthase gene cluster has been completely cloned and characterized, but still a lot needs to be known about the exact regulatory mechanisms of the production of rifamycin, which also happens to be a secondary metabolite. In order to gain insight into the genomic organization of the Amycolatopsis strains, the whole genome sequencing of its wild type strain, Amycolatopsis mediterranei DSM 40773 was done. For dwelling deeper into the analysis, the comparative genomic analysis of the rifamycin producing strains of A. mediterranei was carried out, all of which dedicate a large part of their genomes (approximately 100 kb) to the production of rifamycin B. Seven rifamycin producing strains were taken under consideration. Also, deletion of the essential acyl transferase domain of module 8 was done in order to see its effect on rifamycin biosynthesis. On deleting the acyl transferase domain, an open chain triketide was expected to be produced. This was followed by the analysis of the antibiosis potential of a rifamycin analog, 24- desmethylrifamycin B. For testing the antibiosis potential, this analog was chemically converted into two of its derivatives, namely, 24- desmethylrifamycin S and 24- desmethylrifampicin respectively. This compound was further converted into 24- desmethylrifampicin chemically. These two drugs along with appropriate controls (rifampcin and rifamycin S) were used for testing the antibiosis potential of the above mentioned derivatives of 24- desmethylrifamycin B. The findings presented in this thesis are aimed to provide a clearer understanding of such antibiotic gene clusters, to produce more analogs by fiddling with them using an amalgamation of the techniques from molecular biology and genomics.

Contents

1. Genome assembly and analysis of amycolatopsis mediterranei DSM 40773 2. Comparative genomics of rifamycin producing strains of the genus amycolatopsis 3. Deletion of the acyl transferase domain of module 8 of the rifamycin polyketide synthase gene cluster of amycolatopsis mediterranei S699 4. Activity of rifamycin B analog, 24- desmethylrifamycin B and its derivatives against MDR strains of mycobacterium tuberculosis. Appendix. List of publications.

680. NIKKI KUMARI
Immunobiological Characterization of Pregnant Mare Serum Gonadotropin (PMSG).
Supervisors : Prof. Madan Mohan Chaturvedi and Prof. K. Muralidhar
Th 22146

Abstract

Gonadotropins are glycoprotein in nature. Among placental gonadotropins i.e. human Chorionic Gonadotropin (hCG) and pregnant mare serum Gonadotropin (PMSG), hCG is the most studied and PMSG is least characterized among the group. It exhibits both FSH and LH like activities in heterologous animal species, although it has only LH like activity in equids. The comprehensive biochemical, physiological and immunological characteristics of PMSG are not completely known. Most prominently, the molecular basis for its dual activity is not adequately explained in existing literature. The present study demonstrates the FSH and LH like bioactivities of PMSG in newly designed bioassays in Swiss mice and Holtzman rats. PMSG had been purified from PMS by biochemical methods. The preparations were characterized and analyzed at each step of purification by Spectrophotometry, ELISA specific for PMSG with anti PMSG antibody, Sialic acid estimations, SDS-PAGE, Western blot and by both FSH and LH in vivo bioassays. The present study had achieved purification of PMSG from PMS. It reports for the first time in the world a new isoform of PMSG which retains only FSH-like activity without perceptible LH activity. The importance of this finding for MOET program in buffaloes has been discussed. In order to understand the structure-function relationship of PMSG, different immunological probes against PMSG were also generated and characterized. A competitive and sandwich ELISA to measure PMSG from PMS was further developed with all the raised and characterized antisera. Antiserum to eLH and PMSG could neutralize both the FSH and LH like biological activities of PMSG in female and male Swiss mice, where as it was unable to neutralize the FSH activity. This indicates that probably the presence of single active site in PMSG for both the activities and also that the active site in hMG for the same activity could be structurally different.

Contents

1. Review of literature 2. Generation and characterization of immunological probes against PMSG to enable study of structure-function relationship in the hormone 3. New isoforms of PMSG 4. Generation of polyclonal and monoclonal antibodies against PMSG: A sandwich ELISA for quantitation of PMSG. General summary and future perspective. References.

681. PANDEY (Neeti)
Spodoptera Litura as a Model for Studying the Role of Midgut Bacteria During Intestinal Inflammation in Insects.
Supervisor : Dr. Rajagopal Raman
Th 22142

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. Summary. References, appendix and list of publications.

682. RAWAL (Vagisha)
Antifeedant, Growth Inhibitory and Oviposition Deterrent Activity of Datura Innoxia Mill. (Solanaceae) Against Spodoptera Litura (Fab.) (Lepidoptera: Noctuidae)
Supervisors : Prof A. K. Singh and Dr. Anupam V. Sharma
Th 22140

Contents

1. Historical resume 2. Materials and methods 3. Feeding responses of spodoptera litura towards extracts of datura innoxia 4. Growth inhibitory activity of datura innoxia against spodoptera litura 5. Oviposition deterrent and ovicidal activity of datura innoxia against spodoptera litura. Discussion. Summary. References.

683. ROSHAN KUMAR
Comparative Genomics, Functional and Taxonomic Studies of Bacterial Strains Isolated from Hexachlorocyclohexane (HCH) Dumpsites.
Supervisor : Prof. Rup Lal
Th 22437

Abstract

Hexachlorocyclohexane (HCH) is a chlorinated hydrocarbon that has been used extensively as pesticide. It primarily exists in five stable isomeric forms a- (60-70%), b- (5-12%), g-HCH (10-12%), d- (6-10%) and ε- HCH (3 to 4%). The most important isomeric form is g-HCH which has insecticidal properties. The purification of g-HCH from the HCH produces HCH muck and their unmanageable disposal had led to the formation of HCH dumpsites throughout the world. Interestingly, these HCH dumpsites have revealed the abundance of sphingomonads that can degrade HCH isomers attributed by lin genes. The aim of the thesis was to isolate novel bacterial strains from the dumpsite and deciphering their genomic attributes. Although, several sphingomonad genomes have been sequenced and analyzed, but in order to understand the mechanism of survival of HCH degrading and non-degrading sphingomonads the current study was performed. The genome analysis of a non-degrader of HCH i.e., Sphingobium lactosutens DS20^T revealed the presence of a large number of mobile genetic elements, especially IS6100 in strain DS20^T which corresponds to the high level of genomic rearrangement that could eventually lead to the onset of lin pathway in this strain. Further, the comparative study of nine Sphingobium strains added into the mechanism of survival of these strains at the dumpsite. Till now, there is no suitable method available to monitor the degradation of HCH isomers at these dumpsite. Therefore, a

recently discovered technique; compound specific isotope analysis (CSIA) has been explored to track the fate of HCH degradation. The study reports the first time application of CSIA for enzymatic degradation of a-HCH. Further, the study leads to the characterization of novel bacterial species from the genus Parapedobacter based on genotypic, phenotypic and chemotaxonomic methods and thus the strain was named as Parapedobacter indicus.

Contents

1. Introduction. 2. Genome analysis of HCH non-degrader sphingobium lactosutens DS20^T isolated from hexachlorocyclohexane dumpsite, lucknow, india. 3. Comparative genomic analysis of nine sphingobium genomes: insight into their potential to overcome the environmental stress. 4. Carbon stable isotope fractionation of α hexachlorocyclohexane (HCH) using enzymatic degradation with linA variants. 5. Taxonomical characterization of a novel bacterium isolated from soil contaminated with HCH. Appendices and list of publications.

684. ROSHAN (Rakesh)
Comparative Study on Role of Heat Shock Protein in Evolution of Life History Traits in Drosophila Melanogaster Selected for Faster Pre-adult Development and Late Reproduction.
Supervisor : Dr. Mallikarjun N Shakarad
Th 22136

Contents

1. Introduction 2. Materials and methods 3. Energy reserve and stress tolerance under temperature stress 4. Developmental stability and fitness of progeny after temperature stress 5. Hsp90 expression in response to temperature stress 6. Summary. References. List of Publications.

685. SAGEENA (Geetanjali)
Role of Energy in Oxidative Stress Tolerance Induces by $FeSO_4$, $CdCl_2$ and Paraquat in Drosophila Melanogaster Selected for Divergent Traits.
Supervisor : Dr. Mallikarjun N Shakarad
Th 22441

Contents

1. Introduction 2. Materials and methods 3. Role of juvenile environment in pre-adult development and adult metabolites. 4. Impact of reproduction and aging on heavy metal tolerance 5. Effect of mating on herbicide induced oxidative stress tolerance in drosophila melanogaster selected for divergent traits 6. Effect of heavy metals induced stress on larval and adult behavior 7. Ros scavangers in drosophila melanogaster with reduced internal energy reserves. References and list of publications.

686. SARAAV (Iti)
Functional Characterization and Immunological Evaluation ofMymA Protein of Mycobacterium Tuberculosis.
Supervisor : Dr. Sadhna Sharma
Th 22442

Abstract

Tuberculosis is a global emergency, with ~8 million new cases and ~3 million deaths annually. Limited efficacy of Bacillus Calmette–Guérin (BCG) vaccine and drug resistant Mycobacterium tuberculosis strains has raised the need to identify new vaccine candidates and drug targets in mycobacteria. MymA protein (Rv3083) is a cell wall associated protein involved in modification of mycolic acid in the cell wall of mycobacteria and plays an important role in persistence, thus making it an important drug target. Based on the current understanding of the significance of MymA, its functional characterization was done. For functional characterization of MymA initially bioinformatics studies were performed followed by the biochemical characterization. The present study characterizes for the first time that MymA is a mycobacterial flavin containing monooxygenase and a target of isoniazid. Proteins involved in fatty acid metabolism plays an important role in virulence of mycobacteria so, it is important to study their immunogenic potential. In the present study, mymA operon proteins were analyzed in silico for the presence of HLA class I and HLA class II binding peptides using T cell epitope prediction software. Results demonstrate that of the seven proteins of mymA operon, MymA generated maximum number of epitopes binding to HLA Class I allele and Class II allele. In this study, we also investigated the immunomodulatory role of MymA in macrophages for the first time. Experiments were conducted in THP1 cells and in primary culture of monocyte derived macrophages of BCG vaccinated healthy individuals. Our finding demonstrates that stimulation by MymA result in the activation of macrophages in a TLR2-dependent manner and in the initiation of the adaptive immune responses by polarizing the development of T cells to a Th1 response. These findings indicate that MymA protein can be further investigated as a recombinant BCG/subunit vaccine candidate.

Contents

687. SARKAR (Hironmoy)
Differential Gene Expression in Testes of Wall Lizards and its Comparison with Testicular Gene Expression During Developmental Stages in Mice: Role of CELF Family Proteins in Germ Cell Proliferation and Differentiation Using Transgenic Mice
Supervisor : Prof. Umesh Rai
Th 22664

Abstract

Testes are the sites of spermatogenesis which requires coordination among different cell types as well as tight regulation of gene expression. For a few species, especially for seasonal breeders in a temperate climate, spermatogenesis also dependents on environmental factors like temperature. To compare the gene expression pattern in testes of seasonal and non seasonal breeders, we performed subtractive hybridization

between active and regressed phase from Hemidactylus flaviviridis (Indian wall lizard) and identified differentially expressed genes. These genes were further revalidated in the testes during regressed (pre-meiotic), recrudescent (meiotic) and active (post-meiotic) phases of spermatogenesis by real-time PCR. These gene expression patterns in testes of wall lizard was compared with the corresponding gene expression pattern found in testis of 5 (pre-meiotic), 20 (meiotic) and 60 (post-meiotic) days old mice. Genes like Hk1, Nme5, Akap4, Bco2, Arih1, Rassf7 and Tubb4b were highly expressed in the active phase of lizard testis and in 60 days of mouse testis. On other hand based on the previously published work from our laboratory, we found two members of Celf family (Celf1 and Celf2) were highly expressed in regressed phase of wall lizard testis. CELF family proteins regulate multiple stages of gene expression followed by mRNA maturations. To investigate further the role of CELF family in germ cell division and differentiation, we have generated transgenic mice expressing CelfΔ (suppress the CELF family members) under germ cell specific promoter Vasa. Transgenic mice showed no such aberration in germ cell development as well as in the overall spermatogenesis. This indicates that there might be some alternative mechanism for the observed redundancy in the function of CELF family in germ cells. The similar gene expression profiles in testes of lizard and mice suggests the conserved role of these genes in regulating spermatogenesis despite the evolutionary distance between lizard and mice.

Contents

688. SETHI (Tanu)
Bioefficacy, Evolution and Inheritance of Resistance to Cry1Ac and Cypermethrin in Brinjal Shoot and Fruit Borer, Leucinodes orbonalis (Guenee) (Lepidoptera: Crambidae).
Supervisor : Prof. A. K. Singh and Dr. G. T. Gujar
Th 22141

Contents

689. SHAH SADDAD HUSSAIN
Gonadotropins and gonadal Metabolism: Regulation of Ovarian L-GFulonate-3-Dehydrogenase by Buffalo Pituitary Follicle Stimulating Hormone (buFSH)
Supervisor : Prof. Madan Mohan Chaturvedi and Prof. Kambadur Muralidhar
Th 22439

Abstract

To understand the biochemical mechanisms by which ascorbic acid depletion is caused by LH while its concentration is increased, on the other hand, by FSH, it was tested whether this occurs through modulation of L-Gulonate 3-dehydrogenase (L-GuDH) activity in the ovary. Hence purification and kinetic characterization of the enzyme L-GuDH was undertaken. Further, it was decided to clone and express biologically active buFSH-cDNA. Recombinant buffalo-FSH was expressed in CHO cells by transfecting cDNA clones of both α-and-β subunits in pDUO2 vector. During purification, it was observed that 30-70% saturation with ammonium-sulphate pellet and the pH 7 eluate in ion exchange resin had maximum activity. In order to reduce the cost, Glycoengineered Pichia strain (Mn3GnT2) system was used for expression. α and β subunits of FSH without signal peptide were cloned into the pGAPZαA vector having Kex2 signal site and then expressed. After fermentation and isolation, maximum activity was observed in pH7-eluates from SP-Sepharose-chromatography. While the effect of LH and FSH on the ovarian L-GuDH activity was determined, the kinetic and physiochemical analysis of this enzyme by in vitro and in silico study was undertaken initially. Later using PMSG (for its long plasma half-life) in place of FSH, it was observed that the folliculotropic action includes enhancement of the specific activity of this enzyme activity. However increase in both ascorbate level and L-GuDH activity of the gonad as observed during FSH (PMSG) action indicated that increase in ascorbic acid was not brought out by inhibiting this enzyme. Microarray analysis of ovarian transcripts after PMSG injection followed by pathway analysis revealed that 1904 transcripts including that of Lactone oxidase but excluding that of L-GuDH, showed higher expression whereas 1414 transcripts showed down regulation of expression. It was tentatively concluded that both lactone oxidase and L-GuDH are rate-limiting enzymes in the ascorbic-acid biosynthesis.

Contents

690. SHARMA (Sushma)
Temporal Variation in the Diversity of Soil Diazotrophic Population and nifH Expression of Pseudomonas sp. S10 Under Stress Conditions.
Supervisor : Dr. Dileep K Singh
Th 22137

Contents

conditions 5. Effect of pseudomonas sp. S10 on the diversity and nifH expression of native diazotrophic population in soil 6. Summary. References.

691. SINGH (Ngangbam Sarat))
Enzymatic Studies of Endosulfan and Endosulfan Sulfate Biodegradation by Achromobacter Xylosoxidans C8B.
Supervisor : Dr. Dileep K Singh
Th 22143

Contents

1. Introduction 2. Biodegradation of endosulfan and endosulfan sulfate by Achromobacter xylosoxidans strain C8B 3. Enzymatic study of endosulfan and endosulfan sulfate degradation by Achromobacter xylosoxidans strain C8B 4. In-silico screening of enzymes capable of degrading endosulfan and endosulfan sulfate 5. Conclusion 6. Publication.

692. SINGH (Rashmi)
Effect of Fluoride on Fish Immune System.
Supervisor : Dr. Shibnath Mazumder
Th 22144

Abstract

Fluoride compounds are ubiquitously present in soil, water and food. The relentless anthropogenic activities have led to elevated fluoride levels in environment including aquatic bodies. Fish is considered to be an excellent model to evaluate the effect of aquatic toxicants. However, there is little information pertaining to immuno-toxic effect of fluoride on fish. The aim of the current study was to evaluate the immuno-toxic effect of fluoride on fish immune system with two different model system- Clarias sp. and Danio rerio. Our in vivo studies with Clarias sp. demonstrated chronic fluoride exposure led to significant alteration in serum biochemical profile and histopathology of headkidney, spleen and liver. Fluoride-induces significant cytotoxicity in headkidney and spleen. We observed significant reactive oxygen species generation and abrogation in the phagocytic potential of HKM from fluoride-exposed fish. Moreover, fluoride alters the detoxification machinery in headkidney. Danio rerio was used as the model to deduce the effect of fluoride on immune response related gene. Chronic fluoride exposure affected superoxide dismutase gene expression along with upregulation in caspase-3 expression. It impairs pro-inflammatory cytokines gene expression and renders fish susceptible to bacterial infection as reflected with substantial increase in bacterial load in fluoride-exposed fish. Headkidney macrophage (HKM) from Clarias sp. was used as model to elucidate molecular mechanisms of fluoride cytotoxicity. We observed that fluoride-induces caspase-dependent HKM apoptosis where in calcium signaling cascade plays a critical role in initiating the process. Our results suggested the involvement of both intrinsic and extrinsic caspases in fluoride-induced HKM apoptosis. We conclude fluoride is immuno-toxic to fish and the major mediators in fluoride cytotoxicity are calcium and reactive oxygen species.

Contents

1. Introduction 2. Review of literature 3. Rationale & objectives 4. Objective-1 5. Objective-2 6. Objective-3 7. Conclusion 8. References 9. Appendix.

693. SINGH (Samar Pal)
Study of Water Temmperature and Dissolved Oxygen Stress on Immune System, Digestive Enzyme Profile and Gene Expression in Freshwater Fishes.
Supervisor : Prof. Rina Chakrabarti
Th 22135

Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion 6. Summary and conclusions. References. Publications.

694. SURBHI
Neural Correlates of Seasonal Responses in Songbirds.
Supervisor : Prof. Vinod Kumar
Th 22134

Contents

1. General Introduction 2. Study I: Seasonal plasticity in the peptide neuronal systems: Potential roles of GnRH, GnIH, NPY and VIP in regulation of reproductive axis (A) Subtropical resident Indian weaver birds (B) Temperate migratory redheaded buntings 3. Study II: Seasonal plasticity in the olfactory system 4. Study III: Seasonal neuronal plasticity in the song control system and auditory forebrain areas 5. Study IV: Role of melatonin in the regulation of daily and seasonal responses 6. Summary and conclusions 7. References 8. Research publications and presentations.

695. SURLEEN KAUR
Identification of Molecular Defects in the Granulosa Cells from Women with Polycystic Ovary Syndrome.
Supervisor : Dr. Rita Singh
Th 22147

Contents

1. Introduction and review of literature 2. Objectives 3. Materials and methods 4. Part I: Identification of genetic defects at transcriptional level in granulosa cells from PCOS women 5. Part II: Understanding the metabolic defects in granulosa cells from PCOS women. Supplementary data. Summary and conclusions. References. Publication.

696. TAUQUEER AHMAD
The Impact of Temperature and Dissolved Oxygen Induced Oxidative Stress on Catla Catla and Channa Punctatus and its Mitigation Through Amino Acid and Vitamin Enriched Diets
Supervisor : Prof. Rina Chakrabarti and Prof. B.D. Banerjee
Th 22443

Contents

1. Introduction. 2. Review literature. 3. material and method. 4. Result. 5. Discussion. 6. Summary and conclusion. 7. Refernces. 8. Publications

697. USHA
Endosulfan Induced Alterations on the Immune System of Clarias Gariepinus.
Supervisor : Prof. Neeta Sehgal
Th 22138

Abstract

Endosulfan, an insecticide, induces toxicity in non-target organisms like fish though the mechanisms remain obscure. It is also not clear whether endosulfan undergoes metabolic biotransformation in situ, per se. We investigated the role of headkidney (HK), an important fish immune organ on endosulfan metabolism and the long term effects of endosulfan on fish immune system and disease susceptibility. C. gariepinus were exposed to 2.88 ppb ($1/10^{th}$ LC_{50}) endosulfan for 30 d followed by their maintenance in endosulfan free water for 30 d. Our result documented the presence of both α- and β- isomers of endosulfan along with the toxic metabolite endosulfan sulfate (ESS) and direct relationship between cytochrome P450 1A (CYP1A) expression and ESS levels in the HK. Depuration studies suggested the persistence of ESS in the HK and its interference in significant improvement in the changes induced by endosulfan. We report, chronic endosulfan exposure induces HK pathology, impaired phagocytic and bactericidal potential of headkidney macrophage (HKM) functioning, suppressed T-cell proliferation and serum antibody production by the B-cells. Significant increase in intracellular bacteria, percent mortality and extent of tissue damage in exposed-challenged fish compared to unexposed-challenged fish was also observed, suggesting immunosuppressive role of endosulfan. Collectively, our findings suggest that HK plays important role in endosulfan metabolism. We propose that endosulfan induces the activation of CYP1A in HK which led to generation of persistent metabolite, ESS per se, resulting in immunotoxicity and rendering fish prone to opportunistic infections.

Contents

1. Determination of 96 h LC_{50} of endosulfan for clarias gariepinus 2. Endosulfan induced alternations in the head kidney structure of clarias gariepinus 3. Impact of endosulfan on innate and adaptive immune response clarias gariepinus 4. Impact of endosulfan on host-pathogen interaction between clarias gariepinus and aeromonas hydrophila 5. Endosulfan metabolism in headkidney of clarias gariepinus 6. Impact of endosulfan withdrawal on immune response of clarias gariepinus 7. Nature of endosulfan induced cell death. Summary. Composition and preparation of some common buffers and reagents. Formulae. References.

698. VATS (Tarun Kumar)
Biological Activity of Certain Plant Extracts Against Diamondback Moth Plutella Xylostella (L.) (Lepidoptera: Plutellidae).
Supervisors : Prof. A. K. Singh and Dr. Sanjiv Mullick
Th 22145

Contents