CHAPTER 60

ZOOLOGY

Doctoral Theses

01. BASAK (Reetuparna)
Receptors for Gonadotropins and Sex Steroids in Testis of Spotted Snakehead Channa Punctatus: Expression during Different Reproductive Phases and Under the Effect of Human Chorionic Gonadotropin.
Supervisor: Prof. Umesh Rai
Th 22901

Contents

1. General introduction. 2. General methodology. 3. Seasonality of reproduction in male spotted snakehead channa punctatus: correlation of environmental variables and plasma sex steroids with histological changes in testis. 4. Expression of fonadotropin receptors, fshr and lhr, during different testicular phases and their response to hCG stimulation in spotted snakehead channa punctatus. 5. Differential expression of androgen receptors in testis of spotted snakehead channa punctatus along the reproductive cycle and under the effect of hCG. 6. Identification and expression receptors for female sex steroids, estrogen and progesterone, in testis of spotted snakehead channa puncatus during different reproductive phases and under gonadotropic control. 7. Summary. 8. Appendix. 9. List of publications.

02. BHARDWAJ (Priya)
Analysis of Albumin-Associated Cancer Specific Proteins in Human Plasma for Their Role in Cancer Progression and As Cancer Biomarker.
Supervisor: Prof. Rita Singh
Th 23158

Abstract
(Verified)

The survival of cancer patients can only be increased by screening the blood biomarkers for early diagnosis. Blood plasma is a representative of altered metabolism, neoplastic growth, and status of tumor progression. Due to increased activity of proteases in tumor microenvironment, differential low molecular weight proteins/peptides are generated that could be the rich source for cancer specific diagnostic information and understanding their role in tumor progression. Previously, several cancer specific Brip1 protein's fragments were identified in plasma of cancer patients in our laboratory. Further, to understand the significance of the circulating Brip1 protein/peptides, various studies were undertaken. In addition, the albumin associated proteins in plasma of lung cancer patients were investigated. In this study, we have developed competitive ELISA for the measurement of plasma Brip1 protein and investigated its level in breast cancer (BC) patients. The level of Brip1 protein in plasma was significantly high in BC patients in comparison to normal controls. Also, Brip1 protein was found significantly increased in patients with invasive and high grade tumors, implicating its role in malignancy. The plasma Brip1 protein was found to have higher sensitivity and specificity than the existing breast cancer markers, CEA and CA 15-3. Subsequently, we have investigated the effect of Brip1 on breast cancer cell lines. The results showed that the Brip1 participates in tumor progression by increasing proliferation, migration and invasion processes.

This study suggests that the plasma Brip1 protein could be used as a biomarker for diagnosis as well as potential target for treating the malignancy. Further, the levels of specific proteins were increased in albumin enriched fraction of plasma obtained from lung cancer patients. These specific proteins were found to be involved in biological functions such as cellular movement and molecular transport. The identified proteins also posses the potential for diagnosing lung cancer patients.

Contents

1. General introduction. 2. Hypothesis 3. Materials and methods. 4. Development of ELISA for the detection of circulatory brip1 protein and investigation of levels of plasma brip1 protein in breast cancer patients. 5. To investigate the cancer properties of brip1 peptide in breast cancer cell lines in vitro. 6. To investigate the albumin rich fraction of plasma of lung cancer patients. Summary and conclusions. Future perspectives. References. List of publications.

03. CHONGTHAM ANJALIKA
Testing of Phytochemicals for the Treatment of Neurodegenerative Diseases Using Transgenic Drosophila as a Model Organism.
Supervisor: Prof. Namita Agrawal
Th 22902

Contents

1. Introduction. 2. Materials and methods. 3. Curcumin modulates cell death and is protective in huntington's disease model. 4. Withanolide treatment alleviates huntington's disease pathology in Drosophila. 5. Phytochemical eugenol: a suppressor of neurodegenerative disease in drosophila model. Summary and References.

04. GURJAR (Bahadur Singh)
Design and Use of Diagnostic Approaches, Including Nanoparticles, In Immunological Investigations of Hemolytic-Uremic Syndrome
Supervisor: Dr. Anita Kamra Verma
Th 22904

Contents

1. Introduction. 2. Historical resume 3. Materials and methods. 4. Typical HUS and Stxl ½ genes. 5. Technical comparison in between MLPA, qPCR and standard PCR 6. Cfhr 1/3 gene deletion frequency estimation. 7. Gold nanoparticles (AuNPs) and qPCR. 8. Anti-CFH autoantibodies. 9. Cfhrl knockdown preparation in hep3B cell line. 10. Discussion. 11. Summary. 12. References.

05. JHA (Neelu Anand)
Light Effects on Circadian Clock- Controlled Activity and Singing Behaviours, and Reproduction in Zebra Finch, Taeniopygia Guttata (Vieillot 1817).
Supervisor: Prof. Vinod Kumar & Prof. Sangeeta Rani
Th 22905

Abstract
(Verified)

Most species, if not all, exhibit temporal patterns in their behaviour and life processes, which are synchronized toperiodically changing environment. The present research has attempted to study circadian control of song and singing behaviour in zebra finches (Taeniopygia guttata). The activity, reproduction and cognitive phenotype were also studied. The thesis has been organized in three sections. The experiments in the first section tested the circadian control of singing and activity initially entrained to cyclic 12h light: 12 h darkness (12L:12D) and subsequently subjected to constant dim light (LL_{dim}) and constant bright light (LL_{bright}), as well as tested the effects of absence of the pineal gland on daily singing. The results suggest a role of circadian clock in governing both activity and singing behaviour. In the second section, the effects of non-photic factors like food and social cues on song features and singing were studied. Song was not directly affected by food enrichment, but the female presence restored rhythmicity in singing and activity in males with disrupted circadian rhythms. In the third section, a long term effect of pinealectomy and LL environment on activity and singing, reproduction and associated behaviours, and cognitive phenotype were studied. Both the pinealectomy and LL affected the reproductive performances. Interestingly, LL did not disrupt daily activity and singing patterns over generations. However, song features were negatively affected, and birds had poor singing performance and poor copying of the parent's song under LL than controls. Further, birds performed poorly in associative cognitive tasks and showed negative effects for personality traits under LL. Overall, the results support the hypothesis that zebra finch song is a circadian clock-controlled behaviour. The long term exposure to LL restores rhythmic behaviour suggesting adaptation to the external light environment, although the underlying mechanism remains unknown.

Contents

1. General introduction. 2. General materials and methods: section I: Light and circadian clock control of daily activity and singing behaviours. section II: Role of non-photic environment: effects of food richness and social environment. section III: trans-generational impact of the light environment on reproduction, behaviour and cognitive phenotype 3. Summary. 4. References 5.Publications and presentations

06. KHANDELWAL (Preeti)

Studies on Expression of Estrogen Responsive Genes & Precursor Product Profiling Of Vitellogenin in the Indian Freshwater Catfish, Heteropneustes Fossilis.

Supervisor: Dr. Neeta Sehgal

Th 22903

Abstract
(Not Verified)

Aromatase catalyses conversion of androgens into estrogens. Two isoforms of aromatase, cyp19a (ovarian aromatase) and cyp19b (brain aromatase), and two isoforms of estrogen receptors (ER), erα and erβ, are expressed in different tissues of catfish during ovarian growth phase. Expression of cyp19a, in ovary is responsible for production of estrogen that induces synthesis of vitellogenin in liver. Expression of cyp19b in brain, controls release of gonadotropin from pituitary. ERs expressed predominately in liver and gonads. Estrogen produced by aromatases acts via ER therefore erα and erβ, are co-expressed. Expression of cyp19a, cyp19b, erα, erβ and vtg has been corroborated with ovarian cycle in female catfish. Ovarian aromatase upregulated in the ovary during late preparatory and early prespawning phase resulting in the production of estradiol-17β. Circulating estradiol-17β induces vitellogenin synthesis in liver; therefore vtg expression upregulated. Increase erα expression in ovary and increase in ovarian weight indicates estrogen-signaling via erα is essential for vitellogenesis during preparatory and pre-spawning phases. Up-regulation of erβ expression, maintains ovaries in regressed state during post-spawning phase. Production of estrogen in brain controls the release of gonadotropin from pituitary. Expression of cyp19b during maintenance phase culminates into storage of gonadotropin in gravid females. Common EDCs have been screened to compare their interaction with hERs, using in silico docking method. Although genistein has maximum estrogenic potential as compared to other EDCs assessed, but fails to express vtg transcripts. Genistein is found to be a weak phytoestrogen in catfish, Heteropneustes fossilis. Vitellogenin (Vtg) from serum and lipovitellin (Lv) from egg-yolk extract have been isolated and characterized by gel filtration chromatography. Their Purified peak fractions have been resolved into several bands on SDS-PAGE. Tryptic digests of these polypeptides have been analysed by MALDI-TOF/MS. Presence of conserved domains and identical sequences obtained MALDI-TOF/MS suggest a precursor-product relationship between Vtg and Lv.

Contents

1. Expression of estrogen responsive genes in heteropneustes fossils: tissue-specific distribution, annual profile and on exposure to genistein. 2. Vitellogenin and lipovitellin in heteropneustes fossilis: isolation, characterization and precursor product relationship. 3. Summary 4. References. 5. Publications

07. KHANGEMBAM CHERITA DEVI
Study Of Ammonia Oxidozing Archaea And Bacteria in Recirculating Aquaculture System: Their Composition, Activity And Abundance Of Amoagene
Supervisor: Prof. Rina Chakrabarti
Th 22906

Abstract
(Verified)

Recirculating aquaculture system (RAS) is based on reutilization of aquaculture wastewater using the help of biofitration. Broken earthen pot (BEP) was used as biofilter and the diversity of microbes involved in the biofiltration and their responses to the environmental factors (temperature, dissolved oxygen and substrate concentration) were evaluated. The sequences derived from biofilter showed 99-100% similarity with the members of Nitrosomonadaceae and Thaumarcheota. The archaeal amoA copy numbers was significantly ($P < 0.01$) higher than bacterial amoA copy numbers. Biofilters were exposed to 10, 20, 30 and 40°C for 40 days in synthetic wastewater (2mM ammonium chloride). The highest activity was observed at 30°C, followed by 20, 40 and 10°C. Archaeal and bacterial amoA copy number were also highest at 30°C in both, followed by 20, 40 and 10°C. Effect of dissolved oxygen was evaluated by exposing the biofilters at 1, 3, 5, 7 and 9 mg/l for 30 days. Highest activity was observed at 5 and 7 mg/l followed by 3, 9 and 1mg/l. However, the lowest copy number of archaeal amoA was recorded at 7 mg/l while the treatments were also not significantly ($P > 0.05$) different from the initial concentration. The highest bacterial amoA copy number was recorded was at 3 mg/l treatment while the lowest was recorded at 1 mg/l treatment. These biofilters were also exposed to different substrate concentration of 15, 100, 500, 2000 and 5000 µM for the period of 16 days and the highest activity was recorded at 500 µM followed by 100, 15, 2000 and 5000 µM. The archaeal amoA abundance was also highest at 500 µM followed by 2000, 5000, 15 and 100 µM. Highest bacterial amoA was observed at 2000 and 5000 µM. There was no significant difference ($P > 0.05$) between the final and the initial substrate concentrations.

Contents

1.Introduction 2. Review of literature 3. Study of diversity and abundance of ammonia oxidizing microbes (archaea and bacteria) in the biofilter of recirculating aquaculture system. 4 Effect of temperature on the activity and abundance of archaeal and bacterial amoagene. 5. Effect of dissolved oxygen on the activity and abundance of archaeal and bacterial amoAgene. 6. Effect of substrate concentrations on the activity and abundance of archaeal and bacterial amoagene. 7. Summary and conclusions. References.

08. KOHLI (Puneet)
Field and In-Vitro Assesssment of Hexechlorocyclohexane (HCH) Using Carbon Stable Lsotope Analysis Withal Genomic and Taxonomic Characterization of Bacterial Species Isolated From HCH Dumpsite
Supervisor: Prof. Rup Lal
Th 23054

Contents

1. Introduction 2. Field based in-situ determination of carbon stable isotope fractionation (^{13}C / ^{12}C) in environmental samples from hexachlorocyclohexane (HCH) contaminated dumpsite. 3. Laboratory based in-vitro determination of carbon stable isotope fractionation (13C / 12C) during aerobic degradation of hexachlorocyclohexane (HCH) by sphingomonads. 4. Draft genome sequence assembly of sphingobium ummariense RL3, a fervent hexachlorocyclohexane (HCH) degrader. 5. Taxonomic characterization of algoriphagus roseus spnov, and pontibacter virosus sp. nov. Isolated from a hexchlorocyclohexane (HCH) contaminated dumpsite located at ummari village lucknow, India. 6. Appendices. 7. List of publications.

09. LAKRA (Ruchi)
Deciphering Cross Talk in Signaling Pathways Rgulated by FSH in Granulosa Cells.
Supervisor: Prof. Rita Singh
Th 23160

Abstract
(Verified)

FSH acts on granulosa cells and its role in cross-talk mechanisms with other hormones such as Insulin and IGF-1 is still not well established. IRS-2 adaptor protein is the major intermediate in Insulin/IGF-1 downstream signaling and its regulation has not yet been studied in granulosa cells. Our data showed for the first time the FSH-specific up-regulation of IRS-2 expression in preovulatory granulosa cells. IRS-2 expression was regulated by FSH at transcriptional and translational levels, and by decreased mRNA degradation. Furthermore, blocking of cAMP/PKA showed inhibition in IRS-2 expression by FSH. We also demonstrated the role of FSH-stimulated Sp1 transcription factor in IRS-2 expression. FSH stimulated the Sp1 translocation into nucleus and binding to IRS-2 promoter. Additionally, siRNA mediated knockdown of IRS-2 significantly altered the FSH-stimulated granulosa cell growth. In addition, we also provided evidence for the role of another cross-talk member from Src tyrosine kinase family in FSH-stimulated ovaries and granulosa cells. We showed the presence of Hck in rat ovary and granulosa cells. Interestingly, there was decreased Hck expression in ovary with increase in age of rat and at different stages of estrous cycle. This study suggested specific role of hck in context of follicles development and recruitment. Further, a significant increase in Hck expression was observed in FSH treated granulosa cells. A pharmacological approach was used to inhibit specific pathways in granulosa cells for adenylate cyclase, PKA, PI3K and Akt, however these pathways were not found to be involved in FSH-stimulated Hck expression. Therefore, these results suggest the involvement of alternative pathway or mechanism of FSH involved in Hck up-regulation in granulosa cells. To our knowledge, this work is the first to dissect the critical molecular link between FSH and Hck expression in rat granulosa cells and provide a biologically plausible role in follicle development.

Contents

1. Introduction and review of literature. 2. Hypothesis 3. Aim of the study. 4. Materials and methods. 5. FSH regulates IRS-2 expression though cAMP/ KA pathway. 6.Hck expression and regulation in rat ovary. 7. Conclusion. 8. Supplementary, References, Publication.

10. MAYANGLAMBAM ROJINA DEVI
Interaction Of Different Host Plants With Plutella Xylostella (L.) (Lepidoptera: Plutellidae) and Endoparasitoid Cotesia Plutellae (Kurdjumov) (Hymenoptera: Braconidae), and Learning Behaviour of This Parasitoid to Novel Odours
Supervisor: Prof. Neeta Sehgal
Th 22907

Abstract
(Not Verified)

Effect of different host plants on various fitness parameters of Plutella xylostella and its larval endoparasitoid, Cotesia plutellae was studied under laboratory condition. P. xylostella larvae survived well on all the tested plants except on mustard. Their development was significantly faster on turnip while prolonged development observed on mustard. Significantly heavier pupae developed from cauliflower fed larvae in comparison to mustard and turnip fed larvae. Significantly lower number of eggs was laid on knol-khol in both choice and no choice conditions, but females prefer to lay eggs on mustard under choice condition. Significantly higher parasitization was observed on turnip and cauliflower fed larvae. Although the development time of parasitoid wasps were significantly longer on mustard fed larvae, no significant difference observed in adult emergence. Male-biased parasitoid population observed on turnip fed larvae. The parasitoids reared on cauliflower survived longer showing more glycogen and sugar reserves as compared to those reared on mustard but the protein and lipid reserves of parasitoids were statistically similar on all tested plants. Adult female wasps showed the ability to learn novel odour associated with host and host plants. The innate response of geraniol and citronellol was found to be neutral when tested in T- tunnel olfactometer. They associate the odour very efficiently with host and host plants and responded strongly towards the experienced odour. However, the learned odour was retained in their memory for 2 hours but lost after 4 hours. They were able to detect the experienced odour when mixed with citronellol in different concentrations. The learning was independent of exposure duration rather the first odour perceived during first host encounter was remembered. It was observed that the parasitoid wasps were able to learn two odours at a time but responded strongly towards the first order perceived when both the odours were presented together.

Contents

1.Introduction. 2.Historical resume 3. Materials and methods. 4. Effect of different host plants on oviposition, survival and development of plutella xylostella. 5. Influence of different host plants on parasitization, development, sex ratio and longevity of cotesia plutellae. 6. Quantification of carbohydrate, proteins and lipid contents of cotesia plutellae. 7. Learning behaviour of cotesia plutellae towards novel odours. Discussion, summary, reference,

11. MISHRA (Ila)
Mechanism Of Adaptation For Breeding In Opportunistic, Atypical Photosensitive And Photoperiodic Songbirds
Supervisor: Prof. Vinod Kumar
Th 22908

Abstract
(Verified)

The present research was undertaken to understand at mechanistic level, the adaptation for breeding in songbirds, representing different breeding patterns: the opportunistic zebra finches (Taeniopygia guttata), atypical photosensitive spotted munia (Lonchura punctulata), and photoperiodic redheaded bunting (Emberiza bruniceps) and blackheaded bunting (Emberiza melanocephala). Thus, the thesis has been organized in 3 sections, each included studies on the species characterizing a breeding pattern. In section I, we monitored changes at behavioural and physiological levels in response to the daily timed food availability (4 h in morning or evening, with controls on ad libitum food) in zebra finches maintained under 12 h light: 12 h darkness (12L:12D). The food availability times differentially affected the reproductive performance and offspring 'quality'. Then, we examined in section II, the expression of hypothalamic neuropeptides suggested to be involved in the regulation of gonadal recrudescence. Particularly, we measured hypothalamic expression of gonadotropin releasing hormone (GnRH-I and GnRH-II), gonadotropin inhibiting peptide (GnIH), neuropeptide Y (NPY) and vasoactive intesitinal peptide (VIP) in spotted munia exposed to stimulatory 3L:21D and non-stimulatory 21L:3D photoperiods. The expression of GnRH-I and GnIH, not GnRH-II, paralleled the gonad development state. Also, there were photoperiod-dependent variations in the expression of NPY and VIP. Section III examined transcriptional changes of hypothalamic gene switches in two bunting species, photoperiod induced with different seasonal states (non-migratory or non-breeding; pre-migratory or early photostimulated; migratory or late photostimulate; post-breeding non-migratory or photorefractory state). Differences in mRNA expression levels between acute and chronic long-day exposures suggested differences at the transcriptional level between the initiation and maintenance of the seasonal response. Also, there appeared a conserved tissue-level circadian regulation of the expression of genes coding for the neurotransmitters. Overall, the gene expression patterns were found to be dependent on the seasonal states. These results on transcriptional regulation extend the idea that transitions between photoperiodic states involve circadian clocks.

Contents

1. General introduction. 2. Role of food in controlling reproduction in opportunistic breeding zebra finches 3. Neuropeptide regulation of gonadal recrudescence in atypical photosensitive spotted munia. 4. Regulation of seasonal states in photoperiodic buntings. 5. Concurrent hypothalamic gene expression under acute and chronic long days. 6. Hypothalamic gene expression across photoperiodic states: daily expression patterns of photoperiod-responsive genes in buntings. 7. Daily expression of genes coding for neurotransmitters in central and peripheral tissues. 8. Seasonal life-history dependent changes in daily rhythms of genes coding for neurotransmitters in central oscillators. Summary and conclusions, references, publications and presentations

12. MISHRA (Nalini)

Evolution Of Life History Traits In Drosophila Melanogaster Populations Selected for Divergent Traits: The Role of Parental Age and Stress

Supervisor: Prof. Mallikarjun N. Shakarad

Th 22909

Abstract

(Not Verified)

Parental age is known to effect progeny life history traits and hence fitness. In nature, Drosophila resides on spoiling fruits and they frequently encounter situations of high densities due to limited resource availability, hence selection for faster development might occur and is found to be favoured and rational. There are innumerable studies independently on parental age effects and scramble competition aspects but never a study on combined effect. So, I tried to ascertain the impact of increasing parental age and increased larval density during growth phase (scramble competition) on the life-history traits of progeny, in two different populations of D. melanogaster, one of which is the ancestral control and the other one selected for faster pre adult development and late reproduction. The selected population was equally capable of performing all vital functions despite of having lesser energy reserves which were resultant of reduced development time. The decrease in development time (27.8%) resulted in correlated decrease in fresh weights by 30.3%, lipids by 62%, proteins by 58%, and carbohydrates by 16.7%. There was severe impact of scramble competition on viability that reduced by 34.67%. Scramble competition resulted in decline of fecundity by 17%. Interestingly, there was no effect of parental age on any of the progeny traits. My results are contrary to other studies. The difference in the results might be due to different genotype of the flies used in different studies and or specific maintenance conditions adopted by different laboratories. The findings from my study suggest that if populations are maintained on non-varying diet and environment, perhaps the parental age effects can be eliminated.

Contents

1. Introduction and historical resume. 2. Materials and methods. 3. Effects of parental age and high larval density (scramble competition) on adult progeny fitness. 4. Effects of parental age and high larval density (scramble competition) on progeny macromolecular contents. 5. The relation between parental age and ROS levels in the parents themselves as well as the ensuing progeny. 6. Effects of parental age and substrate quality on pre-adult fitness of progeny. References, list of publications

13. MOSES RINCHUI NGASAINAO
Digestive Physiology of Snow Trout Schizothorax Richardsonii During Ontogenic Development
Supervisor: Prof. Rina Chakrabarti
Th 22910

Contents

1. Introduction 2. Review of literature 3. Embryonic and larval development of various organs during early ontogeny of snow trout schizothroax richardsonii 4. Digestive enzyme Profile of snow trout Schizothorax richardonii during early ontogeny 5. Effect of dietary supplementation of vitamin C and seeds of achyranthes aspera on the survival, growth and physiology of snow trout schizothorax richardsonii 6. Comparative Study of the digestive enzyme activities of snow trout and other important coldwater species mahseer and hill-trout 7. Summary and conclusions. References. Publications

14. NAYYAR (Namita)
Enantioselectivity and Kinetics of Seven Hexachlorocyclohexane Dehydrochlorinase (LinA) Mutants and Genomic and Taxonomic Characterization of Bacteria Isolated From HCH Contaminated Sites
Supervisor: Prof. Rup Lal
Th 22911

Abstract
(Not Verified)

To validate the specificity and versatility of LinA as the potential enzyme for enzymatic bioremediation, the present research work was undertaken, focussing on the enantioselectivity, reaction kinetics and specificity determination of LinA. Seven mutants of LinA2 were designed based on the amino acid differences between LinA1 and LinA2. These mutants were analysed for their enantioselectivity towards alpha HCH and also their kinetic analysis was done to find a best suited variant of LinA for enzymatic bioremediation. Enzymatic bioremediation approach, while looks feasible, requires extensive studies. In this direction, due to the versatile nature and broad substrate specificity, LinA has been proposed to have the potential to be used in enzymatic bioremediation. However affordable and effective bioremediation requires highly specialized enzymes. In addition to this, in order to understand the genome complement of bacteria present at the dumpsite, genome sequencing of one isolate from the dumpsite, Novosphingobium lindaniclasticum LE124^{T}, which was previously reported to possess degrading gene clusters was performed and its genome was announced. The linA gene of the organism was anlysed and it was observed that the organism posseses only one copy of linA. Also in order to further propagate the existing efforts of identifying and classifying microorganisms from the HCH dumpsite, a novel bacterial isolate belonging to the genus Pontibacter was isolated from a HCH contaminated pond sediment situated in the vicinity of the HCH producing factory at Chinhat, Lucknow, India.

Contents

1. Introduction 2. Enantioselectivity of seven hexachlorocyclohexane dehydrochlorinase (LinA) mutants 3. Reaction kinetics and determination of specificity constant (K_{eat} / K_M) of seven HCH dehydrochlorinase (LinA) mutants 4. Genome sequencing of novophingobium lindaniclasticum LE124^{T}, isolated from a hexachlorocyclohexane dumpsite 5. Taxonomical characterization of a novel bacterial strain (pontibacter muscosus PB3^{T}) isloated from a hexachlorocyclohexane (HCH) contaminated pond sediment 6. Appendices. List of publications.

15. NEGI (Vivek)

Community Genomics And Taxonomical Characterization Of Microbes at Hexachlorocyclohexane (HCH) Contaminated Sites.

Supervisor: Prof. Rup Lal

Th 22912

Abstract

(Not Verified)

Metagenomic analysis of pond sediments, evolution and diversity of linA and linB genes, whole genome analysis of Sphingobium lucknowense F2 and taxonomical characterization of Corneybacterium pollutisoli VDS11T were carried out to understand the role of microbial community at the site contaminated or related with HCH. The study revealed the enrichment of genes belonging to bacterial secretory system, aromatic compound metabolism (degradation of chlorocyclohexane, chlorobenzene, toluene, catechol, terephthalate and xylene isomers), two componentsystem and type VI secretory system. This suggests the active role of microbes inhabiting the pond sediment. Also the analysis of two important genes linA and linB that encode enzymes that are crucial for HCH isomers degradation revealed that linA gene is continuously evolving under HCH pressure. The whole genome analysis of the HCH degrader S. lucknowense F2T although did not show any major difference in the lin gene system as compared to existing genes but it reflected the involvement of some other factors/genes that makes this strain a better HCH degrader. In addition, the taxonomical characterization of C. pollutisoli VDS11T, was performed which although is a non-HCH degrader but a HCH tolerant. We did not get any traces of lin genes in this strain reflecting that these strains that do not degrade HCH perhaps assist the HCH degrader community in performing degradation. However further studies are needed to understand precisely the role of such strains.

Contents

1. Introduction. 2. Metagenomic analysis of contaminated pond sediment located by the side of the industry engaged in the production of lindane and comparison of the microbial community with already available metadata from HCH dumpsite. 3. Phylogenetic analysis of the HCH dehdroclorinase (LinA) and HCH haloalkanedehalogense (LinB) enzymes by using metagenomics approaches from the meta data pond sediment and the HCH dumpsite. 4. Genome analysis of a potent HCH degrader sphingobium lucknowense F2^{T} to explore the functional potential of the strain. 5. Characterization of corynebacterium pollutisoli sp. nov., isolated from hexachlorocyclohexane polluted site. 6. Appendices. 7. List of publications

16. NIDHI

Widespread Distribution Of Huntingtin Aggregated and Its Implications in Huntington's Disease Using Drosophila as a Model Organism.

Supervisor: Prof. Namita Agrawal

Th 23159

Abstract

(Not Verified)

Huntington's disease (HD) is a devastating late-onset neurodegenerative disease characterized by progressive motor dysfunction, disturbed circadian biorhythms, sleep-wake disintegration, cognitive decline and dementia, and weight loss. HD is caused by an abnormal expansion of polyQ repeats in the N-terminal region of huntingtin (Htt) protein. Mutant huntingtin protein (mHtt) with longer polyQ adopts an abnormal conformation and accumulates in the cells as soluble forms at the time of disease onset and insoluble mHtt aggregates/inclusion bodies as disease progresses. The mHtt aggregates have been extensively marked at sites of neuronal degeneration in the central nervous system of HD patients and therefore, are considered as the "pathological hallmark" of the disease. However, recent studies with more sensitive measures suggest soluble mHtt, instead of mHtt aggregates, as the most toxic species in HD. Therefore, a detailed understanding about the potential role of mHtt accumulation in disease pathogenesis in in vivo condition is warranted. Therefore, using transgenic Drosophila that harbours

human huntingtin gene as a model system, we targeted mHtt expression in different neuronal populations that displays disease symptoms in HD such as circadian pacemaker neurons, motor neurons and mushroom body neurons. By targeting expression of mHtt in specific neurons, I found that with the passage of time as mHtt sequesters in the form of aggregates along with the diffused form, it damages these neuronal populations. Interestingly, I observed correlation of the size of the aggregates with the death of the neurons, bigger the aggregates more propensity of cells towards death. With these observations, in present thesis, using an in vivo condition, I clearly showed that aggregates do play important role in neurodegeneration. Bigger aggregates along with diffused form confers neurodegeneration and thus, contribute towards the exacerbation of HD pathology. Thereby, targetting aggregates could be a possible mechanism towards suppression of HD pathogenesis.

Contents

1. Introduction. 2. Toxic effects of mutant huntingtin accumulation on PDF positive circadian pacemaker neurons in drosophila. 3. Effects of mutant huntingtin protein accumulation on neuromuscular junctions in drosophila. 4. Mutant huntingtin accumulation in mushroom bodies impels learning and memory defects in drosophila. Summary and conclusions, References.

17. PANWAR (Deepak)
Studies on Protein- Protein Interactions Regulating Granulosa Cell Apoptosis in Bubalus Bubalis
Supervisor: Prof. Neeta Sehgal
Th 22913

Contents

1. Introduction 2. Review of literature 3. Molecular characterization and interaction of KGF-KITLG proteins in bubalus bubalis 4. Effect KGF and KITLg on apoptosis in buffalo granulosa cells. 5. Summary and conclusions. Bibliography. Publications.

18. PRIYAM (Manisha)
Study on Reptitian Pattern Recognition Receptors with an Emphasis on TLRs in the Wall Lizard
Supervisors: Dr. Soma M. Ghorai & Prof. Umesh Rai
Th 23055

Abstract
(Verified)

Present study was designed to explore the molecular aspects of reptilian immunology in the purview of evolution of pattern recognition receptors (PRRs), particularly toll-like receptors (TLRs). We constructed an immunome for the squamate Hemidactylus flaviviridis from its splenic transcriptome. From the immunome, we obtained nine PRRs, DEC205/ lymphocyte antigen 75 (ly75), Nucleotide-binding oligomerization domain-containing protein 1, NOD-like receptor family CARD domain containing 3 (NLRC3), nucleotide-binding oligomerization domain, leucine rich repeat containing X1 (NLRX1), DDX58/ retinoic acid-inducible gene 1 (RIG-1), TLR3, 4, 5 and 7 with potential full length coding sequences. Evolutionary analysis showed a high degree of conservation of PRR orthologues in vertebrate phylogeny. We assessed the effect of Pseudomonas aeruginosa-induced infection on splenic transcriptome wherein screening of significantly upregulated immune-related transcripts suggested mediation of essentially complement pathways and pattern recognition mechanisms in combating bacterial infection. TLR pathway related transcripts (TLR2, MyD88) were considerably represented in this cluster; therefore we analysed on its expression and tissue distribution in control and infected samples. Significant upregulation of MyD88 and TRIF in infected samples suggested the existence of both MyD88 dependent and independent pathways in wall lizard. Considerable enhancement was seen in expression level of TLR2, 4, 5, 7 and 13 in liver, spleen and ovary, indicating their role in antibacterial response. Overall tissue distribution pattern of TLRs seemed to be similar across vertebrates, wherein spleen exhibited maximal levels of six TLRs (TLR2, 3, 4, 5, 7 and 13) and adapter molecules, MyD88 and TRIF. Finally in this study, we tried to distinguish between selection constraints on lipoprotein sensing TLR 4, 5 and nucleic acid sensing TLR3, 7 wherein the former emerged as the more divergent class. Overall, this is the first intensive study on reptilian TLRs and it paves way for bridging the gap in comparative immunology with regards to reptiles.

Contents

1. General introduction 2.Exploring the lizard immunome: de novo sequencing of splenic transcriptome and clustering of immune-relevant genes. 3. Study on effect of pseudomonas aeruginosa-induced infection on the splenic transcriptome of wall lizard; an insight on antibacterial response of TLRs. 4. Divergence of Lipoprotein sensing (TLR 4,5) and nucleic acid sensing (TLR 3,7) within the reptilian lineage. 5. Summary. 6. Appendices. 7. Publication

19. RASHMI KUMARI
Genetic Manipulation, Comparative Genomics and Taxonomical Characterization of Actinobacteria
Supervisor: Prof. Rup Lal
Th 22914

Abstract
(Not Verified)

Actinobacteria constitute one of the largest phyla among bacteria that includes the most important antibiotic producers (streptomycetes, Amycolatopsis, Salinospora), some deadly human pathogens (Mycobacterium tuberculosis, M. leprae) and plant pathogens (Streptomyces scabies, Leifsonia xyli). Interestingly, some of these bacteria are equipped with an extraordinary capability of producing wide variety of secondary metabolites while others do not produce any valuable secondary metabolite, hence, they are of less importance from both ecological and biomedical perspectives. The traditional or secondary metabolite producing actinobacteria are characterized by a large genome size due to the presence of large number of biosynthetic gene clusters while the non-traditional actinobacteria possess relatively smaller genomes, suggesting that they do not undergo the complex developmental stages. Most of the antibiotics in clinical use today have been isolated from members of this phylum, however, with constant increase in the number of drug-resistant bacteria, there is an increasingly high emaphasis of research to discover new antibiotics. While several strains of actinobacteria are the causative agents of deadly diseases, tuberculosis (TB) has been a rampant health threat worldwide and with time, the situation has deteriorated due to the emergence of multi-drug resistant strains of Mycobacterium tuberculosis that causes TB. The existing drug rifampicin, a semisynthetic derivative of rifamycin B, produced again by an actinobacterium Amycolatopsis mediterranei has been ineffective against MDR-TB. Therefore, to tackle the problem of wide emergence of drug resistant forms of tuberculosis, present study was designed with an aim to develop novel analog of rifamycin B by employing combinatorial biosynthesis approach. Therefore, this study also required in-depth analysis of the genomes of the genus Amycolatopsis which was achieved through comparative genomic analysis. Additionally, a non-traditional actinobacterium belonging to the genus Tessaracoccus was taxonomically characterized, sequenced and also analysed for better understanding of the differences among traditional and non-traditional actinobacteria.

Contents

1. Introduction. 2. Swapping of acyltransferase domain of the fifth module (AT5) of rifamycin polyketide synthase (PKS) gene cluster of amycolatopsis mediterranei S699 with acyltransferase domain of the second module (AT2) of rapamycin PKS gene cluster of streptomyces hygroscopicus for the production of novel rifamycin B analog. 3. Comparative genomic analysis of 29 strains of genus amycolatopsis and an insight into their ability for the production of secondary metabolites. 4. Taxonomical characterization of a novel actinobacterium isolated from the drainage system of lindane – producing factory. 5. Draft genome sequence of tessaracoccus

flavus RPI^T and comparative genomic analysis of the genus tessaracoccus revealing niche specific adaptations. 5. Appendix. 6. List of publications.

20. ROY (Alivia)

RNA-Seq Analysis of Testis During Different Reproductive Phases in Spotted Snakehead Channa Punctatus: Expression Analysis of Jag 1, Notch1, Kpna 2, Sstr2, Klhl10, Gria1, Arnt2, Foxj1, Plzf And Thx8 Along the Testicular Cycle and Under the Effect of Human Chorionic Gonadotropin

Supervisor: Prof. Umesh Rai

Th 22915

Abstract

(Verified)

The present Ph.D. work presents a consolidated picture of in silico analysis, reproductive-phase dependent temporal expression and gonadotropic regulation of a few testicular genes implicated in regulating seasonality of testicular cycle in spotted snakehead Channa punctatus. Testicular transcripts annotated and classified under various subcategories of biological process, molecular function and cellular component showed highest number of transcripts during spawning phase for majority of the subcategories. In addition, an appreciable increase in upregulated transcripts of various biological processes was observed during spermatogenically active phases. Also, differentially expressed transcripts relevant to spermatogenesis were identified. Out of these, jag1, notch1, kpna2, sstr2, klhl10, gria1, arnt2, foxj1, plzf and lhx8 were selected for further analysis. The structural domains were identified in the predicted protein sequences and their phylogenetic trees were constructed following in silico analysis. Based on expression profile of these genes along the testicular cycle, we speculated that ligand-receptor pair jag1 and notch1 are involved in spermatogonial stem cell (SSC) renewal and onset of spermatogonial differentiation, plzf in SSC renewal, lhx8 in spermatogonial differentiation and release of spermatozoa, kpna2 and klhl10 in spermiogenesis and release of spermatozoa, klhl10 and foxj1 in restructuring of seminiferous lobules and apoptosis of germ cells, arnt2 in homeostasis of spermatogonial cells, and sstr2 and gria1 in causing testicular regression. With regard to gonadotropic regulation, human chorionic gonadotropin (hCG) was found capable in modulating the expression of jag1, notch1, kpna2, sstr2, klhl10, gria1, arnt2, foxj1, plzf and lhx8 in C. punctatus. Interestingly, the expression pattern of these genes after 3, 5 and 14 days of hCG treatment was largely comparable to the pattern observed from resting to postspawning phases. It is noteworthy that this Ph.D. work for the first time reports temporal expression along the testicular cycle and gonadotropic regulation of the selected testicular genes in teleost testis.

Contents

1. General introduction. 2. General methodology. 3. De novo sequencing and comparative analysis of testicular transcriptome from different reproductive phases in freshwater spotted snakehead channa purctatus. 4. In silico analysis, seasonal variation and gonadotropic regulation of jag1 and its receptor notch1 in testis of spotted snakehead channa punctatus. 5. Expression pattern of kpna2, sstr2, klhl10 and grial in testis along the reproductive cycle and under the effect of human chorionic gonadotropin in spotted snakehead channa punctatus. 6. Identification, temporal expression and gonadotropic regulation of transcription factors arnt2, foxj1, plzf and lhx8 in testis of seasonally breeding spotted snakehead channa punctatus. 7. Summary. 8. Appendix. 9.List of publications

21. SHRIVASTAVA (Nitisha)

Repurposing of Pharmacopoeia For Identifying Novel Radioprotectors Using Zebrafish as Organism Model .

Supervisor: Prof. Neeta Sehgal

Th 22916

Abstract
(Not Verified)

The study deals with finding novel modulators of radiation injury using zebrafish as organism model. Amelioration of radiation induced injuries were assessed as end points in zebrafish embryo to find novel leads from John Hopkins Clinical Compound Library (JHCCL). Two small molecule, viz. ethynodiol diacetate (ED) and scopolamine methyl bromide (SMB) were identified from the library as radiomodulators in pre and post radiation scenario. ED was further evaluated under 30 days survival assay in murine model. ED exhibited 66.6% and 33.3% survival advantage respectively in pre and post radiation scenario. In vitro tumor sensitization studies performed with ED exhibited DMF (dose modifying factor) of 0.86 in A549 cell line. In vivo sensitization assay revealed 3 fold reduction in tumour volume and growth delay of 11 days. A 0.53 log cell kill was observed with ED and 15 Gy. ED also exhibited 9 days delay with a 0.24 log cell kill as a chemotherapeutic agent. In silico studies were done to assess functional targets that were modulated, succumbing radiation injury. A ChIN model was adopted and developed in the embryos to study immune markers of inflammation viz. neutrophils and macrophage. Semi-quantitative gene expression studies for inflammatory enzymes and cytokines were performed after radiation injury at two time points (6 hoursand 24 hours). Both the molecules were majorly found to modulate inflammation acting as a glucocorticoid receptor agonist (ED, Binding Energy (B.E) -9.0 Kcal/mol) and Cox-2 antagonist (SMB, B.E -8.0 Kcal/mol). ED also has antioxidant potential (0.26 and 0.93 fold reductions in DCFDA fluorescence and MDA absorbance respectively) and SMB has anti-apoptotic potential (6% reduction in sub G1 population, 1.19 folds reduction in caspase-3 levels). These results suggest that ED and SMB are potential candidates that can be further evaluated in higher mammals for amelioration of ionising radiation induced injuries.

Contents

1. Review of literature. 2. Introduction. 3. Material and methods. 4. Results. 5. Discussions. 6. Summary and conclusions, references , publications, presentation and awards, appendix

22. SINGH (Neetika)
Studies on Epigenetic Regulation of Anti-Proliferative Property of Curcumin on Hela Cells, in vitro
Supervisor: Prof. Madan Mohan Chaturvedi
Th 23161

Abstract
(Not Verified)

Histone and non-histone proteins form a highly compact and a dynamic polymer called as chromatin. Post-translational modifications of histones play a crucial role in alteration of the conformation of the chromatin, which govern various DNA-dependent processes. Further, these modifications are dynamically regulated by various histone-modifying enzymes which require several metabolites as their cofactor/co-substrates. Such an intricate correlation between metabolites and histone-modifications proposes a mechanistic link between cell's metabolic state and epigenetic regulation. Food is a conventional way to modulate the level of various metabolites, which in turn positions food as an upstream regulator to the histone modifications. Curcumin (diferuloylmethane), a dietary phytochemical has been extensively reported to play a vital role in cancer prevention and /or therapy. Its potential beneficial effects can be attributed to its ability to induce various epigenetic alterations, by bringing changes in DNA methylation, histone modifications and expression of miRNAs. In the present study, epigenetic regulation of anti-proliferative property of curcumin in cervical cancer HeLa cells was studied. Results indicated that curcumin induced dose and time dependent cytotoxicity (MTT assay and light microscopy) and apoptosis (DNA fragmentation, AO/EB staining, FACS analysis, caspases activation, expression of tumor suppressor proteins, such as p53 and p21) in HeLa cells. Further, global post-translational histone modifications, such as H3K9ac, H3K4me3, H3K9me3, H3K36me2, H3S10P were also studied in curcumin treated cells with the help of western blotting and immunocytochemistry, using site- and modification- specific histone antibodies. The apoptotic histone code comprising of five different post-translational modifications revealed a characteristic pattern (increase in H3K9ac, H3K9me3, H3K4me3 and H3K36me2; parabolic in H3S10ph) and has been hypothesized to push a cell's fate from

cell-survival to cell-death. The findings are suggestive of a cross-talk mechanism between various histone modifications which might be due to the up/down regulation of various genes, involved in apoptosis.

Contents

1. Introduction. 2. Materials and methods. 3. Results. 4. Discussion. 5. Summary. References.

23. SINGH (Swati)

Immunological Profile of Latency Associated DosR Regulon Antigens RV 2626c and Rv2032 of Mycobacterium Tuberculosis

Supervisor: Dr. Sadhna Sharma

Th 23056

Abstract
(Not Verified)

Tuberculosis remains a great health threat to the world particularly with the advent of HIV and emergence of drug resistant strains. According to WHO approximately 1/3rd of the world's population is latently infected with Mtb which is at the highest risk of transmitting TB. Targeting latently infected individuals to permanently control or completely eradicate the infection therefore becomes the immediate urgency of present times. Latent infection is associated with expression of 48 DosR regulon genes which are being studied as stage-specific potential vaccine candidates. By bioinformatics studies using software NetMHC 3.4, BIMAS, IEDB, ProPred, Vaxijen, Hex and CABSDOCK, Rv2626c and Rv2032 were selected as probable T cell antigens and pursued for further immunological analysis. Recombinant proteins were synthesized in bacterial system and used to stimulate PBMCs derived from 20 BCG vaccinated healthy subjects, 20 TB patients and 20 healthy contacts of TB patients to assess the kind of immune response produced by these antigens. In our studies on human PBMCs, these proteins were seen to trigger a beneficial response against TB by increasing the frequency of $CD4^{+}/CD8^{+}$ memory cells and $CD4^{+}/CD8^{+}$ mediated IFN-g release; by augmentation of expression of transcription factor T-Bet which is the master regulator of Th1 cytokines; by upregulation of Th1 cytokines IFN-g and IL-2 and down regulation of Th2 cytokines IL-10 and TGF-b and by down modulation of Treg and Th17 cells in TB patients and their contacts. The study also glimpsed into the interplay between various T cells and cytokines and broadened our perspective of protective response beyond just $CD4^{+}$ T cells and IFN-g production. We have shown that the 2 latency associated proteins Rv2626c and Rv2032 produced strong in-silico as well as experimental evidences of protective and immunogenic response. Therefore, we suggest that these could be further analysed as potential post-exposure vaccine candidates against TB.

Contents

1. Review of literature 2. Aims and objectives: objective-1. identification of putative latent T cell antigens associated with mycobacterium tuberculosis objective-II. In-vitro evaluation of recombinant Rv2 626c And Rv2032. 3. Summary and conclusion. 4. List of publications

24. SINGH (Khushboo)

Soil Microbial Diversity Using 16S rDNA and Biodegradation of Lindane by Achromobacter Sp. Strain NWA6c1

Supervisor: Prof. Dileep K. Singh

Th 22917

Abstract
(Not Verified)

Lindane (γ-HCH) is highly chlorinated and has been extensively used as a broad-spectrum insecticide. Microbial degradation offers an effective and environmental approach to remove such pollutants from the environment. The first objective of the present study is to have an insight of temporal variations in various physico-chemical parameters and HCH isomers in the wastewater and soil of Najafgarh drain, Delhi. Under this, physical parameters varied significantly throughout the sampling period and the range of the parameters were found to be above the restriction limits concluding that the drainage water is not suitable for irrigation or any other purposes. The pesticide analysis showed that the water and soil samples contained varied concentration of HCH isomers. The mean ΣHCH in water varied from 1.24-1.91ppm whereas ΣHCH in soil varied from 1.09-1.47 ppm. The result exhibited the presence of HCH residues in most of the samples because of their continued industrial manufacture and unauthorized use by the farmers. The presence of HCH might also be due to the result of their prolonged persistence in the environment. In the second objective, temporal changes in the bacterial communities were observed using 16S rDNA analysis throughout the sampling period. We employed DNA based T-RFLP technique to reveal the variability of microbial communities in presence of different environmental factors. The study showed the presence of different bacterial groups known for their tolerance of different pollutants. In the last objective, our work was to isolate, identify the Lindane degrading bacteria from the polluted site of Najafgarh drain. Achromobacter sp. strain NWA6c1 was isolated by the enrichment technique from the Najafgarh drain soil. Degradation of Lindane under optimized conditions (pH 7.0, temperature 30ºC and 50ppm γ-HCH) was found to be significantly enhanced by the isolated strain.

Contents

1. Introduction. 2. Review of literature. 3. Temporal variations in various physico-chemical parameters and HCH isomers in the wastewater and soil of Najafgarh drain, Delhi. 4. Temporal variations in the diversity of 16S rDNA of Najafgarh drain soil. 5. Isolation, identification & optimization of lindane degrading bacteria for its enhanced degradation and invitro study of lindane induced alteration in the total RNA yield. 6. Summary. 7. References

25. TRIPATHY (Mamta)

Studies on Epxression and Gonadotropic Regulation of Gonadotropin and Female Sex Steroid Receptors, Aromatase and Growth Factors in the Ovary of Wall Lizard, Hemidactylus Flaviviridis.

Supervisor: Prof. Umesh Rai

Th 23162

Abstract
(Verified)

The Ph.D. work in Indian wall lizard, Hemidactylus flaviviridis for the first time provides a comprehensive picture of molecular mechanisms associated with ovarian folliculogenesis and their gonadotropic regulation in reptiles. Sequence characterization of putative amino acid sequences of selected proteins (FSHR, PR, BMP1, 2, 3, 3b, 7, 15 and GDF9) revealed similarity of major domains, functional motifs and residues with that of respective human orthologs, indicating conservation of receptor-ligand interactions and signaling. Phylogenetic analyses showed that all the putative proteins of wall lizard analyzed in the current study were closer to that of other squamates, except PR which was closer to mammals. Further, expression of a few selected genes (fshr, cyp19, er-α, er-β, pr, bmp15, gdf9) was quantified in the ovary of wall lizard during different reproductive phases to correlate with follicular development along the reproductive cycle. Following qPCR, high ovarian expression of gdf9 and er-α, and moderate level of fshr during regressed phase suggest their prime role in oogonial proliferation, maintenance of germinal bed, formation of stromal primordial follicles and development of tiny extrastromal follicles. High expression of ovarian bmp15, fshr, ers (er-α and er-β), cyp19 and increased plasma E_2level coincident with development of previtellogenic follicles during recrudescence suggest the involvement of BMP15, FSH and estrogen in proliferation and differentiation of polymorphic granulosa cells and development of thecal layer. Expression pattern of pr in ovary and plasma level of progesterone along the reproductive cycle of wall lizards showed a wider role of progesterone, from folliculogenesis to ovulation, egg retention and oviposition. In vivo experiment with FSH corroborates our assumptions based on temporal expression pattern of the selected genes and follicular development in the ovary. Further, in vitro experiment established the direct role of FSH in regulation of these genes in ovary of wall lizards.

Contents

1. General introduction. 2. General methodology 3. Sequence characterization, temporal expression and gonadotropic regulation of follicle stimulating hormone receptor in the ovary of wall lizards. 4. A correlation between ovarian expression of aromatase, estrogen receptors and plasma estradiol during different reproductive phases of wall loizard hemidactylus flaviviridis: their gonadotropic control. 5. In silico analysis of ovarian progesterone receptor and correlation of its temporal expression with plasma progesterone depending on reproductive phases and under the effect of gonadotropin in Indian wall lizard hemidactylus flaviviridis. 6. Repertorie of bone morphogenetic proteins and growth / differentiation factors in ovary of wall lizards with emphasis on differential expression and gonadotropic regulation of bmp 15 and gdf9. Summary. Appendices (I-IX).

26. WADHWA (Neerja)
Autologous Transplantation of In-Vivo Genetically Modified Spermatogonial Stem Cells
Supervisor: Dr. Soma M. Ghorai
Th 22919

Abstract
(Not Verified)

Chemotherapy and radiation treatments for cancer can permanently damage the fertility. Adult male patients have an option to preserve their future fertility before gonadotoxic therapy by cryopreserving sperm. Unfortunately, in pre-pubertal boys, the only option to preserve fertility is by cryopreservation of SSC that can be obtained via testicular biopsy prior to gonadotoxic therapy.These cells can be autologously transplanted into their testes after cure. Hence, autologous transplantationis the actual clinical requirement to treat male infertility. For a successful autologous transplantation, we have established a novel and efficient method for generating germ cell depleted testis in a short duration of time (15 days) using direct testicular busulfan injection without causing any cytotoxic effect in other organs. This can substitute the commonly used intra-peritoneal method.Next, pre-pubertal mouseSSC were genetically modified by in-vivo testicular electroporation by inserting pCXEGFP gene in one of the testis of mice. Transgenic SSC were isolated and expanded in-vitro from a single electroporated and cryopreserved testis. Till date, the isolation and expansion of SSC has been possible from a pool of mice testes. EGFP transfected SSC were transplanted into the busulfan treated contra-lateral testis. Donor-derived germ cell colonies in the testes of busulfan treated recipients showing EGFP expression at 2 months post transplantation indicated successful autologous GCT. Pem-TSPAN8-IRES2-EGFP transgenic mice were generated using testicular electroporation method. Various in-vivo andin-vitro studies confirmed that TSPAN8 increases stem cell renewal and germ cell proliferation and is useful in enhancement of cell numbers. The feeder layer with TSPAN8 expression would be useful for enhancement of SSC number from limited resources and efficient autologous transplantation in pre-pubertal oncological patients. The knowledge generated in this study may offer significant translational benefits for treating male infertility in pediatric oncological patients who suffer from the gonadotoxic side effects of radiation and chemotherapy.

Contents

1. Abbreviations. 2. General Introduction. 3. Objectives and scope. 4. Chapter 1: autologous transplantation of in-vivo electroporated spermatogonial stem cells. 5. Chapter-II: use of tetraspanin 8 (TSP AN8) for enhanced self-renewal and proliferation of spermatogonial stem cells (SSC) for efficient germ cell transplantation from limited SSC. 6. Summary. Bibliography. Appendices. Publications.

27. VERMA (Vipin Kumar)

Immuno-Stimulatory Effects of Supplemented Feed Against Aeromonas Hydrophila and Development of ELISA to Evaluate Health Status of the African Catfish, Clarias Gariepinus and the Murrel, Channa Punctata.

Supervisor: Dr. Om Prakash & Prof. Neeta Sehgal

Th 22918

Abstract

(Not Verified)

Many bacteria cause infection to fish and these infections can also get transferred to humans. The bacteria isolated from various fish tissues were identified as associates of Providencia alcalifacien, Myroides odoratimimus, Myroides odoratus, Myroides phaeus, Proteus vulgaris, Proteus mirabilis and few strains of Morganella morganii. The teleost fish have an advanced immune system and can produce immunoglobulins to neutralize the antigens. The molecular weights of purified immunoglobulins (IgM) from Clarias gariepinus and Channa punctata was determined to be 840 kDa and 820 kDa, respectively in native form. The molecular weight of heavy chain of IgM was estimated to be 74.5 kDa in C. gariepinus and 72.5 kDa in C. punctata. In this study primary antibodies were raised in rat against BSA and heavy chain of immunoglobulin to develop Sandwich ELISA (for estimation of antigen specific antibody) and Indirect ELISA (to estimate total IgM), respectively. These antibodies also showed cross reactivity with immunoglobulins of various fish belonging to different order. Methanol and ethanol extracts of aerial root of F. benghalensis and pod seed of L. leucocephala showed the presence of total phenol, total flavonoids and exhibited antibacterial and antioxidant properties. The GC-MS of methanol extracts of these plants showed the presence of various biologically active compounds. Fish fed on 5% supplemented feed didn't exhibit any change in the SGOT, SGPT and ALP levels suggesting, no liver or kidney damage. The supplemented feed also decreases the stress (Increase in SOD and decrease in LPO levels), enhanced the innate (increase in phagocytosis, lysozyme and nitric oxide levels) and adaptive (increase in immunoglobulins levels) immune response in C. gariepinus and C. punctata when challenged with A. hydrophila. So this study advocates the use F. benghalensis & L. leucocephala supplemented feed to enhance fish immune response.

Contents

1.Isolation &characterization of pathogenic bacteria from marine and freshwater fish. 2. Isolation, purification and characterization of immunoglobulin from clarias gariepinus & channa punctata and development of ELISA for its estimation. 3. Immuno – modulatory role of artificial feed supplemented with ficus benghalensis and leucaena leucocephala in clarias gariepinus and channa punctata. 4. Summary. Literature cited. Publications.