CHAPTER 59

ZOOLOGY

Doctoral Theses

01. ANINA JAMES

Degradation of Atrazine by Epiphytic Root Bacteria Isolated from Emergent Hydrophytes and Impact of Atrazine on Protein Profile and Total RNA of the Isolates.

Supervisor : Prof. Dileep Kumar Singh

Th 23987

Abstract
(Not Verified)

Atrazine is a herbicide used in several countries world over besides India. It is known to be highly persistent, potentially carcinogenic and is an endocrine disruptor. The present study aims to identify bacteria and plant species capable of decontaminating atrazine. Epiphytic root bacteria were isolated from emergent hydrophytes *Acorus calamus*, *Typha latifolia* and *Phragmites karka*. Gas chromatography of growth medium inoculated with strains ACB, AACB, PKB, PPKB, TLB and TTLB, showed a reduction in amount of atrazine (AACB showed maximum reduction-88% of 10 mg l) through 15 day exposure. In LAM (liquid atrazine medium) and LB, the optimum pH for all the strains for decontaminating 10 mg l atrazine was 7. Optimum temperature for the strains AACB and TTLB lay between 30 to 40°C and for strain PPKB, 20 to 30 °C. *A. calamus*, *T. latifolia* and *P. karka* with their associated root bacteria *Pseudomonas* sp. strain ACB, *Pseudomonas* sp. strain TLB and *Arthrobacter* sp. strain PKB could remove environmentally relevant concentrations of atrazine hydroponically. Plant growth promoting traits of the isolates aided the hydrophytes in overcoming atrazine stress. The cell free extracts of strain AACB showed decontamination of 90 % of the added atrazine in a span of 5 hours. Peptide sequence of band 1 of strain AACB has homology with the peptide fragment of CoA ester lyase of *Sphingorhabdus flavimaris*. Peptide sequence of band 1 of strain ACB has significant homology with peptide fragment of alkyl hydroperoxide reductase of *Oleiphilus* sp. There was a reduction in the intensity of RNA bands of both strains ACB and AACB when they were treated with atrazine. Studies such as these lay the foundation of information on which we have to build a strong strategy for bioremediation of atrazine, in situ and ex situ.

Contents

1. Introduction. 2. Review of literature 3. Isolation and identification of epiphytic root bacteria from emergent hydrophytes acorus calamus, typha latifolia and phragmites karka 4. Effect of variation in physical parameters like pH and temperature and nutrition and atrazine decontamination by the isolates 5. Assessment of atrazine decontamination by the emergent hydrophyte-bacterium associations and in vitro screening of the isolated for plant growth promoting potential 6. Differential expression of proteins and variation in total RNA yield of proteins and variation in total RNA yield of the isolates under the impact of atrazine 7. Summary. Publication.

02. BAJAJ (Swati)

Biodegradation of γ–Hexachlorocyclohexanae (Lindane) by free and Immobilized Halophilic Bacteria Isolated from HCH Dumpsite and Induced Expression of ABC Transporter Proteins.

Supervisor : Prof. Dileep Kumar Singh

Th 23973

Abstract

(Not Verified)

Large dumps of unused γ-hexachlorocyclohexane (γ-HCH) have become an issue of concern. The overall salinity of soil enhances due to the release of potassium cations by the insecticide in the polluted soil, which may limit microbial degradation. The current work addresses this problem through the isolation of γ-HCH degrading halophilic bacteria from HCH dumpsite using γ-HCH as a sole carbon source by selective enrichment technique. The isolated bacteria, which were identified as Chromohalobacter sp. (KU934224.1), Halomonas sp. (MF776947.1), and Marinobacter sp. (KY744745.1) on the basis of 16SrRNA gene sequence analysis, can rapidly degrade -HCH (50 mg L-1) at various salt concentrations. The highest degradation was attained at 5% NaCl under aerobic conditions. Low amount of metabolites like 1,2,4-trichlorobenzene (1,2,4-TCB) and 2,5-dichlorophenol (2,5- DCP) were identified during the degradation. Lindane degrading halophiles were tested for their ability to degrade lindane upon immobilization. Cells of halophilic strains immobilized on calcium alginate were found to have better lindane degradation activity than the ones, which were immobilized on agar-agar or free cells. The study assessed the reusability of the alginate-immobilized cells during three periods of 7 days each without much loss of the activity suggesting their good application prospect. The expression of ABC transporter proteins in halophiles in the presence of lindane suggests that they are probably involved in the transport of lindane inside the cell and clearance of dead-end metabolites that are toxic to the cell. The results have described the ability of moderately halophilic bacteria to use lindane as the sole carbon and energy source. The study demonstrated the participation of cellular factors for the utilization of lindane in halophilic strains. The strains have not been previously shown to grow and degrade γ-HCH and might be good candidates to remediate saline environments contaminated with γ-HCH.

Contents

1. General introduction. 2. Review of literature 3. Isolation and identification of γ–HCH degrading halophilic bacteria from HCH dumpsite and derivation of the possible degradation route 4. Whole cell immobilization of bacteria and its effect on lindane degradation under varying environmental conditions 5. Study the effect of lindane on bacterial protein profile and morphology 6. Summary. Bibliography and publication.

03. DUGGAL (Shweta)

Delineating the AKT 1-Dependent Cell Cycle Regulatory Signalling Networks.

Supervisors : Prof. Namita Agrawal and Dr. Kanury V. S. Rao

Th 23979

Abstract

(Not Verified)

Cell growth and proliferation are two diverse processes yet always linked. There is a complex interplay between various signalling mechanisms functioning all through the early phase of the cell cycle. The curiosity in studying the signalling pathways that regulate cell

proliferation increased substantially when it became evident that these pathways were altered in numerous forms of cancer. Akt1, a serine/threonine kinase, is a multi-functional protein implicated in regulation of cell growth, survival and proliferation and is one of the known proto-oncogene products that has been found to be mutated in many cancers. The precise mechanism by which Akt1 regulated the cell cycle, and also the manner in which it coordinated cell growth and proliferation – however - remained unclear. Accordingly, it was hypothesized that a resolution of the protein-protein interactions that Akt1 engages in, and an understanding of how such interactions are modulated as cells progress through the cycle, should shed some light on this question. Therefore the present study was carried out to characterize the Akt1 interactome, and also to capture the dynamics of its interactome as the cell progresses through the cell cycle. To decipher the path from Akt1 that mediated cell cycle regulation, a cell cycle specific interactome for Rb protein was also delineated. This study provides the first experimental description of Akt1 and Rb interactomes with their alterations in composition during cell cycle progression. The results reveal some novel proteinprotein linkages through which Akt1 executes its function in driving cell division. A subset of proteins were identified as intermediaries in establishing a link between Akt1 and Rb protein Such studies should shed more light on processes governing cell proliferation and, more specifically, the molecular mechanisms by which Akt1 executes its influence over this process by recruiting the key cell cycle regulatory protein Rb, and its binding partners.

Contents

1. Review of literature 2. To delineate the interacting partners of Akt1 and determine the topological changes that occur in this network as function of progression of cells through the different stages of the cell cycle 3. To capture the cell cycle stage specific interacting partners of Rb & define the Rb-centric protein-protein interaction network 4. To integrate the Akt1-specific networks with Rb in order to extract the Akt1-dependent signalling axis that drives the cell cycle 5. Summary and conclusion. Publications.

04. GUISUIBOU DAIMEI

Interaction of Groundnut Bud Necrosis Virus (GBNV) and its Vector Thrips Palmi Karny.

Supervisor : Prof. Rajagopal Raman

Th 23980

Abstract
(Not Verified)

Tospoviruses are exclusively transmitted by several species of thrips in a persistent and propagative manner causing huge economic loss to several crops and ornamental plants worldwide. Several studies have reported the effect of tospoviruses on the fitness parameters of their thrips vector but with contradicting results. Furthermore, the mechanism of *Tospovirus* movement from the midgut to the primary salivary glands in the thrips is not well understood. Thus, this study attempted to understand the interaction of *Groundnut bud necrosis virus* (GBNV) and its vector *Thrips palmi*. The first part describes the effect of GBNV on the life history traits and feeding preference of *T. palmi*. The result showed that pre-adult mortality, adult longevity and fecundity were not affected. However, the developmental period of GBNVinfected thrips was shorter than the healthy thrips. Further investigation on feeding choice assay showed that *T. palmi* preferred GBNV-infected plants than the healthy plants. The second part of this study examined the progression of GBNV-infection in thrips and its transmission. Acquisition access period was given to three

developmental stages of thrips: first instar larvae, second instar larvae and adults. Localization study showed the detection of viral protein in the midgut, tubular salivary glands and primary salivary glands (PSG) of second instar larvae, pupae and adults, if GBNV was acquired during first instar larval stage. However, no infection was observed in the PSG if virus acquisition occurs during second instar larvae or adult stage. Regarding virus transmission, only the emerging adult that acquired the virus during first larval instar can transmit the virus. The wide host range of GBNV also prompted us to look for GBNV infection in ornamental plants, which led to the finding of natural infection of GBNV in *Catharanthus roseus* for the first time.

Contents

1. Introduction. 2. Review of literature 3. Objective 1: To study the influence of groundnut bud necrosis virus (GBNY) on the life history traits and feeding preference of its vector thrips palmi karny 4. Objective 2: To study progression of groundnut bud necrosis virus (GBNV) infection in the development stages of insect vector thrips palmi karny and its transmission 5. Objective 3: Prevalence of groundnut bud necrosis virus (BNV) infection on periwinkle 6. Summary. Publications.

05. HIRA (Princy)
Comparative Genomic Analysis Uncovers the Genomic Heterogeneity and Distinctive Plant Growth Promoting Potential of Pseudomonas Fluorescens and Bradyrhizobium Sp.
Supervisors : Prof. Mallikarjun Shkarad and Prof. Rup Lal
Th 23983

Contents

1. Introduction – (Part-I): Complete genome sequencing and comparative genomic study of Pseudomonas fluorescens spp.: Insights into their genomic heterogeneity, colonization strategies and plant growth promoting potential. (Part-II): Complete genome sequencing and analysis of native isolates of Bradyrhizobium yuanmingense R33 and R34 to decipher the evolution and nodulating capability comparable to the introduced strain from USA Bradyrhizobium diazoefficiens USDA110 2. Summary. Appendices and list of publications.

06. JAIN (Punita)
Understanding the Role of Situins in Autophagy Using Dictyostelium Discoideum as a Model System.
Supervisors : Prof. Anju Shrivastava and Prof. Shweta Saran
Th 23977

Abstract
(Verified)

Sirtuins, class III histone deacetylases are evolutionary diversified group of proteins, which are present across the taxa from eubacteria, archaea to eukaryotes. Sirtuins in various lower organism regulate cellular metabolism and promotes cellular survival leading to organismal lifespan extension. But ongoing conflicts related to SIRT1 as aging regulator has allowed us to delineate the potential role of Sirtuin *in Dictyostelium discoideum.* We conducted our present studies in the lower eukaryotic model D. discoideum, a unicellular haploid amoeba. There are five Sirtuins present in this organism, which allow us to understand their key functions in autophagy induction and its possible involvement in lifespan extension. Here, nutrient signaling

triggers multicellularity and cell death takes place independent of apoptosis. We have analysed two (out of the five known) Sirtuins: Sir2A and Sir2D. Functional analysis revealed that overexpression of sir2D like its other homologs, promote cell survival by extending chronological life span, cell viability, and autophagy flux, whereas deletion of the same leds to developmental defects like multi-tipped phenotype, altered cAMP signaling accompanied with cell death. We found reduced autophagic flux in the sir2D- cells, which was increased upon overexpression of sir2D suggesting that *DdSir2D* is not only involved in growth and development but also modulates the levels of autophagy. Besides this we also demonstrated Sir2D as a key, though not the sole regulator of AMPK and TOR signaling pathway. Also, deletion of sir2A (homolog of human SIRT2) showed impaired cell proliferation, G0/G1-phase arrest, deceased cell viability with altered cAMP levels and developmental defects. sir2A- cells showed a clear accumulation of autophagosomes and reduced autolysosome. Although, sir2A modulates autophagy levels but partial redundancy due to other Sirtuins still remains to be investigated. Hence, the knowledge gained from both sir2A and sir2D help us to understand the evolutionary conservation of Sirtuins in *D. discoideum.*

Contents

1. An Introduction to sirtuins and dictyostelium discoideum 2. Elucidating the role of sir2D in growth, development and starvation-induced autophagy 3. Functional analysis of sir2A in dictyostelium discoideum 4. Effect of resveratrol on growth, development and autophagy-induction 5. Dictyostelium discoideum as a model system to study autophagy-mediated lifespan extension. Summary and conclusions, references, appendix A and B.

07. JOSE (Anisha)
Antigen/Antibody Detection in Plasma of Cancer Patients.
Supervisor : Prof. Rita Singh
Th 23978

Abstract
(Not Verified)

We evaluated the diagnostic efficacy of circulating human Brip1 protein and its autoantibody in cancers of breast, ovary and lung by using our in-house developed indirect ELISA system. We measured plasma Brip1 protein and autoantibody levels of cancer patients including 169 breast cancer patients, 23 patients with ovarian cancer and 54 lung cancer patients and their respective patients with benign conditions and healthy controls. Plasma levels of circulating Brip1 protein were significantly higher for breast cancer, ovarian cancer patient and lung cancer than for patients with benign conditions and healthy controls. Plasma Brip1 protein had a sensitivity of 95.24% and a specificity of 92% in separating breast cancer population from all other individuals. The Brip1 assay was significantly more sensitive and specific than CA15.3 assay in breast cancer detection. Moreover, Patients with cancer of breast, ovary and lung have significant higher circulating levels of anti-Brip1 autoantibodies as compared to control subjects ($P < 0.0001$). The study found that the test discriminates breast cancer patients with the benign and healthy controls with a high sensitivity of 92.23% and a specificity of 95% than for other cancer patients included in the study. The results have demonstrated that Brip1 can induce a relatively higher frequency of autoantibody response in breast cancer compared to the cancer of ovary and lung. The frequency of Brip1 expression in breast cancer tissues was significantly higher than that in adjacent normal tissues ($P <0.01$). In conclusion, ELISA is a simple and reliable method to

measure the diagnostic potential of circulating Brip1 protein and its autoantibody in plasma of cancer patients and play a role in the development of a multi-marker assay for detection of cancer.

Contents

1. Development and validation of blood based indirect elisa for quantification of circulating brip1 protein in human plasma of different cohort of cancer patients 2. Identification and validation of brip1 autoantibodies in blood plasma. Conclusion, future direction, references and publication.

08. KHAN (Nawaz Alam)
Physiological Responses of Indian Major Carps Catla Catla and Labeo Rohita to Different Light Intensities and Role of Dietary Supplementation of Vitamin C, Vitamin E and Tryptophan in Stress Amelioration.
Supervisor : Prof. Rina Chakrabarti and Prof. Jai Gopal Sharma
Th 23974

Abstract
(Not Verified)

Environment plays an important role in the life of aquatic organisms. Light intensity is a key limiting environmental factor that elicits differential responses in fish at various developmental stages. The effect of light intensity and stress ameliorating role of vitamin-C, vitamin-E and tryptophan on *Catla catla* and *Labeo rohita* was evaluated in the present study. Fish were exposed to five different light intensities of 983 ± 162 lx, 1828 ± 324 lx, 2676 ± 409 lx, 3442 ± 648 lx and 114 ± 4 lx (ambient light considered to be control) for 90 days under photoperiod of 12L: 12D. In one set of experiment, fish were fed with vitamin-C, vitamin-E and tryptophan enriched diets and in another set they were fed with control diet without any enrichment. An inverse relationship was found between the light intensity and survival rate, average weight and specific growth rate (SGR) of the fish. Significantly ($P < 0.05$) higher SGR were recorded in control fish cultured under ambient light of 114 ± 4 lx compared to the exposed fish. High light intensity caused physiological stress in fish which was reflected by drastically elevated levels of serum cortisol and glucose. This was further proved by the increased levels of heat shock proteins at higher light intensities. Exposure of fish to higher light intensity resulted in oxidation of lipids and proteins as malondialdehyde and carbonyl protein levels were significantly ($P < 0.05$) higher in fish exposed to high light intensity. Glutathione peroxidase, glutathione S-transferase and swimming activities also showed the similar trend. Dietary supplementation of vitamin-C, vitamin-E and tryptophan ameliorated the harmful effects caused by high light intensity. Care should be taken during the time of aquaculture, particularly during the larviculture. The information generated from this study may be useful for the aquaculture industry.

Contents

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

09. KUMARI ADITI
Study of Metabolic Activity in Neurodegenerative Diseases using Transgenic Drosophila as a Model System.
Supervisor : Prof. Namita Agarwal
Th 23984

Contents

1. Introduction. 2. Materials and methods 3. Neuronal expression of mutant huntingtin impairs lipid metabolism in drosophila model of huntington's disease 4. Neuronal expression of mutant huntingtin (mHTT) affects reproductive ability in drosophila 5. Phytochemicals curcumin and tinospora modulate body weight and metabolic activity in drosophila model of huntington's disease. Summary, References and list of publication.

10. MAHATO (Nitish Kumar)
Characterization of Bacterial Diversity and Metabolic Dynamics from Himalayan Geothermal Springs Located at Manikaran, Himachal Pradesh.
Supervisors : Prof. Yogendra Singh and Prof. Rup Lal
Th 23985

Abstract
(Verified)

Geothermal springs being one of the major reservoirs of thermophiles and hyperthermophiles. Metagenomics analyses along with genomics and comparative genomics can provide significant insights into (i) discovery of yet-unknown bacterial genotypes and (ii) genetic and functional rationale behind thermophilic adaptations. Manikaran geothermal springs located atop the Himalayan ranges at Manikaran in Kullu (Himachal Pradesh) being the hottest (temperature ~96℃) geothermal springs across the country were selected to analyze microbial community dynamics explicitly involving the characterization of bacterial diversity and exploring their metabolic potential using metagenomic, genomic and comparative genomic analyses. Physicochemical analyses showed prevalence of heavy metals. Community profiling of geothermal water metagenome revealed abundance of *Proteobacteria*. Functional profiling of geothermal water samples reflected enrichment of complete biosynthesis pathways and genes coding for thermophilic proteins. Advanced computational tools were used to reconstruct the genome of a novel thermophilic ecotype *Emticicia* sp. strain MM from shotgun metagenomics sequence data and compared with the mesophilic reference available of the same genus. Culture-based techniques enabled isolation, genome sequencing, *de novo* genome assembly and genome analysis of a moderately thermophilic strain *Deinococcus* sp. strain RL from sediments of Manikaran geothermal springs. Presence of 14 CRISPR arrays having 169 spacers indicated enhanced rate of bacteriophage invasions. Further, comparative genome analysis of 29 genomes of the genus *Deinococcus* revealed distinct phylogeny of all representatives and evolution of habitat-specific traits of strain RL. The core genome (n=959 genes) and pan-genome (n=16,883 genes) were identified. Key reasons for enhanced genome shuffling in these isolates were their active natural competence machinery. Thus, the extensive studies have not only presented a glimpse of bacterial diversity and their functional potential present in geothermal springs but also have provided a platform to further investigate the geothermal springs using genomics, comparative genomics, and metagenomics to better understand the microbial community dynamics.

Contents

1. Metagenomic analyses of microbial community present in water samples collected from terrestrial geothermal springs, manikaran 2. Genome reconstruction of emticicia sp. MM from metagenomics data and understanding its metabolic potential 3. Draft

genome sequencing and analysis of a moderately thermophile deinococcus sp. stain RL isolated from sediments of geothermal springs 4. Comparative genome analysis of deinococcus sp. strain RL with its closest neighbours to show genome plasticity I extreme environment. Appendices and list of publications.

11. Minhas (Vidisha)
Expression and Characterization of Recombinant Immunogens Inhibiting Fertility in Mice.
Supervisor : Prof. Rita Singh and Dr. Satish Kumar Gupta
Th 24287

Abstract
(Not Verified)

To design a vaccine with improved contraceptive efficacy for management of wildlife population, recombinant proteins comprising either N- (1-80 aa) or C-terminus (76-126 aa) fragments of mouse sperm protein-17 (Sp17; TT-KK-Sp17N, TT-KK-Sp17C) were expressed in *E. coli*. Subsequently, mouse equatorin (21-185 aa) was also expressed (bRNase-KK-EQ). With a premise that fusion of equatorin and Sp17 fragments will enhance the immunogenicity and contraceptive efficacy, two fusion proteins were cloned and expressed in *E. coli* (Sp17N-EQ and Sp17C-EQ). Active immunization of female FVB/J mice with above proteins adsorbed on alum showed high antibody titres against the respective proteins. Highest *in vivo* infertility was observed in TT-KK-Sp17C immunized group. Additionally, pramel1 was also cloned and expressed in *E. coli.* Female FVB/J mice immunized with bRNase-KK-PR1 showed strong immune response and also significant reduction in fertility. However, bRNase-KK-PR1 immunized male FVB/J mice did not result in significant antibody titre and reduction in fertility. Subsequently, C-terminus fragment of Sp17 was expressed as fusion protein with tandem repeat of GnRH either at N- or C-terminus (Sp17C-GnRH2 or GnRH2-Sp17C) in *E. coli*. Sp17C-GnRH2 was more immunogenic than GnRH2-Sp17C. Interestingly, mating studies of female mice with the male mice wherein both were immunized with Sp17C-GnRH2 led to 100% block in fertility. Male mice immunized with Sp17C-GnRH2 led to testicular atrophy and a significant decrease in the diameter and circumference of seminiferous tubules. Further, to reduce the number of injections, group of female FVB/J mice were immunized with Sp17C-GnRH2 emulsified with squalene-arlacel A, which resulted in 90% infertility. Studies following one injection schedule revealed that the group of female mice immunized with a combination of soluble Sp17C-GnRH2 along with Sp17C-GnRH2 entrapped in PLA-MP showed highest antibody titres and percentage infertility as compared to others. The contraceptive efficacy in all groups lasted for 150 days after the single injection.

Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Results and discussion: Chapter 1 5. Results and discussion: Chapter 2 6. Results and discussion: Chapter 3 7. Summary 8. Bibliography 9. List of publications 10. Full publication.

12. PURI (Akshita)
Genomic and Functional Studies of Paracoccus Sp. Stain AK26 Isolated from Hexachlorocyclohexane Contaminated site: Insights into Multipartite Genome Structure and Secondary Metabolites Biosynthesis.
Supervisor : Prof. Yogendra Singh and Rup Lal
Th 23981

Abstract
(Verified)

In the present study a bacterial strain *Paracoccus* sp. AK26 isolated from Hexachlorocyclohexane contaminated soil was identified and characterized. This strain was found to synthesize biologically active isoprenoid-based compounds, *viz* carotenoids and ubiquinone, and carbon storage polymers, polyhydroxyalkanoates. Phylogenomics methods complemented the traditional polyphasic approach to validate the classification of strain AK26 as a novel species within the genus *Paracoccus.* Detailed *in-silico* genome analysis revealed the metabolic capability of strain AK26 to survive in the prevailing environmental conditions and to utilize various carbon compounds (C1-C6) which can be modulated to produce secondary metabolites. Codon usage bias analysis showed that genomic GC content is a key factor affecting the evolution of codon usage in GC rich genomes of the genus *Paracoccus,* thus leading to mutational biases. Further, the complete pathway for the synthesis of characteristic coloured pigment, which was identified as carotenoid type biomolecule, was mapped. Likewise, the pathway for synthesis of ubiquinone CoQ10 and polymeric compound PHAs was also mapped and detailed comparative analysis of the biosynthetic pathway genes revealed the variations in functional and structural organisation of the genetic clusters among the genus *Paracoccus*. The integration of *in-silico* genome analysis for pathway mapping and analytical tools for the characterization of metabolites using various chromatographic and spectroscopic methods provided a comprehensive understanding about the metabolic potential of this bacterium to produce biomolecules. The study also provided the basic framework for developing further strategies to optimize the production using the biosynthetic gene clusters identified in strain AK26.

Contents

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

13. SAGAR (Sneh)
Uptake of Heavy Metals (Ni and Pb) and its Effect on Protein Profile of Rhizobacteria Klebsiella Sp. 10KN.
Supervisor : Prof. Dileep Kumar Singh
Th 23976

Abstract
(Not Verified)

Rapid and huge industrialization has led to the increase in discharge of contaminants like heavy metals, Ni and Pb in waste water and irrigation with this water has declined the quality of agricultural soil. This practice has encouraged searchingfor new possible ways of Ni and Pb detoxification viamicrobes. The current work emphasizesisolation of rhizobacteria from the roots of *Acorus calamus* grown in Ni and Pb enriched waste water.The isolated bacteria, *Klebsiella* sp., 10KN (KU934222.1) can uptake Ni and Pb with great efficiency (Ni 76.2% and Pb 72.4%) examined by MP-AES with facilitating adaptation to extreme environmental conditions (pH, Salinity and temperature). This uptake efficiency was demonstrated qualitatively using microscopical investigations to provide an insight into the uptake mechanism employed by *Klebsiella* sp., 10KN. Tools such as SEM-EDX, FTIR, AFM and TEM analysis demonstrated the morphological adaptation in strain under Ni and Pb stress. Enhanced uptake mechanism of Ni (89%) and Pb (86%)was demonstrated using cultured beads of *Klebsiella* sp. 10KN. Ni and Pb

recovery percentage was also quantified by immobilized biomass of *Klebsiella* sp. 10KNin continuous system of use and reuse.Induced expression ofperiplasmic binding protein and transporting ATPase in the presence of Ni and Pb respectively provided the background of uptake mechanism.PGPR activities like ACC deaminase, Phosphate solubilisation, IAA, Siderophore, HCN and ammonia production shown by rhizobacteria *Klebsiella* sp. 10KN elaborate its potential use for detoxification of toxic elements in agricultural practices and waste waterto sustain its interaction with plants in rhizospheric zones.Present investigations on uptake of Ni and Pb could be helpful in removal, recovery and remediation strategy of heavy metals from the contaminated site using active or immobilized biomass of strain *Klebsiella* sp. 10KN for the betterment of ecosystem in economical manner.

Contents

1. General introduction 2. Review of literature 3. Isolation, identification and characterization of rhizobacteria isolated from roots of the weedy plant acorus calamus efficient in heavy metals (Ni and Pb) uptake 4. Study the effect of heavy metals INi and Pb) on rhizobacterial protein profile 5. Effect of heavy metals (Ni and Pb) on plant growth promoting activities 6. Summary. Bibliography

14. SINGH (Asmita)
Microbial Diversity of Saline Soil and Biodegradation of DDT by Halotolerant Bacillus Subtilis Sp. Stain BGSC1A70 and its Protein Profiling.
Supervisor : Prof. Dileep Kumar Singh
Th 23982

Abstract
(Not Verified)

Modernization of agricultural method has increased wide and production of variety of non-synthetic and synthetic organic chemicals. In spite of ban in many countries including India highly persistent Organochlorine Pesticide like DDT is still being used for pest control at wide range. It is being reported that only approx. 1-2% of pesticide used for pest control reaches the target pathogens and remaining get mix with soil and water. DDT is most wellknown pesticide. In this study microbial diversity from saline soil sampled from sambhar and kozhikode was studied and DDT toxicity and to isolate and identify most efficient halophile bacteria which is able to degrade DDT to significant level and find the specific enzymes which involved in biodegradation of DDT by selected strain was studied. The whole studied was divided into 4 objectives and 6 chapters. Chapter 1 is introduction chapter 2 is Review of literature Chapter 3 is microbial diversity study of saline soil Chapter 4 is Isolation and identification of DDT degrdaing Halophilic Bacteria Chapter 5 is Change in protein profiling of bacteria under DDT stress Chapter 6 covers isolation and purification of protease enzymes Summary References.

Contents

1. Introduction. 2. Review of literature 3. Assessing microbial diversity of saline soil in sambhar lake and Kozhikode 4. Isolation and identification of DDT degrading halotolerant bacteria from saline soil of sambhar lake and Kozhikode 5. DDT, DDD and DDE induced alteration in bacterial protein profile in 1D and 2D page and total RNA yield 6. Purification and characterization of protease enzyme isolated from bacillus subtilis strain BGSC1A70 along with its production and stability under various stress conditions. Summary and references.

15. SINGH (Priya)

Rifamcycin Polyketide Synthase Gene Cluster Analysis, its Genetic Manipulation for Production of Rifamycin Analogs and Proteomic Analysis of Amycolatopsis Mediterranei.

Supervisor : Prof. Rup Lal

Th 23986

Abstract
(Verified)

Each year, over one million people die and nearly 150-200 people die every hour due to TB. There has been a drastic rise in the cases of rifampicin-resistance (RR-TB) and multidrugresistannce (MDR-TB). Rifampicin, semisynthetic derivative of rifamycin B has proved to be cornerstone of the first-line anti-TB regimen. Rifamycin B is produced polyketide synthase (RifPKS) of *A. mediterranei.* The present study focused on understanding the genome attributes & *rif*PKS of rifamycin derivatives producer strain *A. mediterranei* S955. Its draft genome was sequenced, assembled, annotated and deposited in the NCBI/EMBL/GenBank database with the accession no. JMQG00000000 and is ~10.2 Mb represented by 104 contigs with GC content of 71.3% and 9,382 coding sequences and it was found that it has non-sense mutation in *orf*14 gene encoding truncated C-27-O-methyltransferase. The second and third part includes genetic manipulations of strain S699, targeted at the production of desmethylrifamycin by individual swapping of acyl transferase (AT) domains of module 7 and 8 with that of rapamycin PKS and inactivation of dehydratase (DH) domain of the module 10 was done by swapping it for production of 17-hydroxyrifamycin B leading to the generation of mutant clones showing peaks for predicted analogs in LC-ESI-MS data and DH10 mutants showed better activity. Lastly, to gain insights into the reasons why *A. mediterranei* produces antibiotic and what benefit does the organism draw out of it, a study of the complete expressed proteome at two time points (42h and 96h) to decipher the regulation of the rifamycin biosynthesis was carried out and data was obtained using the ThermoLTQOrbitrapVelosHPLC-nESI-LIT-Orbitrap. 2,449 proteins were identified and quantified as compared to 9,575 CDS. Further, *in-silico* protein-protein interactions were done and whole genome PPI showed clustering of the early growth phase proteins and stationary phase proteins in two major clusters interacting through some key regulators.

Contents

1. Genome sequence and rifamycin gene cluster analysis of rifamycin derivatives producer A. mediterranei DSM 46096/S955 2. Swapping of acyltransferase domain of module 7 and 8 (AT7 and AT8) of rifamycin polyketide synthase (rifPKS) gene cluster of A. mediterranei S699 with acyltransferase domain of module 2 (AT2) of rapamycin (rapPKS) gene cluster of Streptomyces hygroscopicus for the production of novel rifamycin B analogs 3. Inactivation (Swapping) of dehydratase domain of module 10 (DH10) of rifamycin polyketide synthase (rifPKS) gene cluster of A. mediterranei S699 for the production of novel rifamycin B analog 4. Protein expression analysis of A. mediterranei S699 at different phases of growth during fermentation. Summary, appendices and list of publications.

16. SOOD (Utkarsh)

Complete Genome Analysis of a Taxonomic Outlier, an Environmental Isolate of Pseudomonas Aeruginosa CR1 and its Comparative Analysis: Insights into Virulence Factors Probable Drug Targets, and Secondary Metabolites.

Supervisors : Prof. Mallikarjun Shakarad and Prof. Rup Lal

Th 23975

Abstract
(Verified)

Outlier strains of *Pseudomonas aeruginosa* are exiguous when compared with classical strains and are either clinical isolates or their source of isolation is not reported. In this study the first complete genome of an outlier environmental strain of *P aeruginosa* CR1 was sequenced and comparative genomic analysis was performed to understand the differences in environmental and clinical outlier strains. Eleven genomic islands were found in strain CR1 imparting strainspecific features to the strain. A plasmid with a VirB/D4 complex followed by trimeric autotransporter was exclusive to strain CR1, and can induce virulence phenotype. Virulence genotype analysis revealed that strain CR1 lacked haemolytic phospholipase C and D, three genes for LPS biosynthesis and had reduced antibiotic resistance genes when compared with clinical strains. Genes belonging to proteases, bacterial exporters and DNA stabilization were found to be under strong positive selection linked with pathogenicity and survival in the outliers. The outliers had the complete operon for the production of vibrioferrin, a siderophore present in plant growth promoting bacteria. The competence to acquire multidrug resistance and new virulence factors makes these strains a potential threat. However, we identified major regulatory hubs that can be used as drug targets against both the classical and outlier groups. The analysis of volatile metabolome of CR1 revealed that it produced pyocyanin and 1-hydroxyphenaxine as major secondary metabolite. The concentration of pyocyanin was determined to be 2.99 µg/ml. Pyocyanin produced by CR1 was bactericidal and also restricted biofilm formation by potent biofilm forming strains of *Bacillus,* therefore it can be harnessed as a bio-pesticide. Hence, the use of *P. aeruginosa* strains as an inoculant in the field should be restricted as they possess a wide arsenal of pathogenic proteins and the ability to acquire multidrug-resistance making them difficult to treat in case of infection to different hosts.

Contents

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