CHAPTER 37

MICROBIOLOGY

Doctoral Theses

01. DATT (Shyama)
Characterization of Virulence Genes of Trichophyton Rubrum and Trichophyton Mentagrophytes from Dermatophytosis Patients.
Supervisors : Prof. Dr. Shukla Das and Dr. S. N. Bhattacharya
Th 25320

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02 DATT (Thakur)
Molecular Characterization of Extended Spectrum β-Lactamase, AMPC β-Lactamase, New Delhi Metallo β-Lactamase and Carbapenemase Producing E. coli Strains Isolated from Patients with Urinary Tract Infection or Diarrhea.
Supervisors : Dr. N. P. Singh, Dr, Iqbal Singh and Dr. Simrita Singh
Th 25318

Abstract
(Not Verified)

E. coli accounts for the large majority of naturally acquired UTI and enteric diseases. It develops resistance to antibiotics by four mechanisms: production of enzymes, Mutations at the binding site, Porin loss, and Efflux pumps. Aim: Molecular Characterization of Extended Spectrum β-Lactamase, AmpC β-lactamase, New Delhi MetalloCarbapenemase producing *E. coli* strains isolated from patients with Urinary Tract Infection or DiarrheaObjective -1. Isolation of *E. coli* strains from UTI and diarrheal patients & Identificationof MDR *E. coli* among these isolates. 2. To study antimicrobial susceptibility testing results of the above *E. coli* strains. 3. Amplification of antimicrobial genes; ESBL, AmpC β-Lactamase, NDM and Carbapenemase of *E. coli* by multiplex PCR. 4. To study AMR variation in the above strains of *E. coli* isolated from patients of UTI with the existing database of *E. coli* strains from UTI cases and among *E. coli* strains isolated from stool of diarrheal patients with the existing database of *E. coli* strains from diarrheal cases. 5. To assess phenotypic expression of Efflux pump and the relative transcription rates of a component of trimeric drug Efflux pumps (tolC), genes that encode membrane porins (ompC&yiaT), a drug resistance-related membrane protease (ompT) and most commonly circulating Carbapenem gene by Real-Time PCR in MDR *E. coli* strains isolated from UTI or diarrheal patients.Methods- MDR *E. coli* isolates isolated from urine and stool were picked-up for detection of AST, ESBLs, AmpC and Carbapenemases phenotypically and molecularly by multiplex-PCR.Results: Potent drugs: Fosfomycin, Tigecyclin, Colistin, Cloramphenicol& Nitrofurantoin.Prevalent genes circulating: CTX-M, CMY & NDM. one isolate carrying CTX-M+TEM+SHV genes.33.93% in urine and 34.38% diarrheal stool MDR *E. coli* isolates co-harbor CTX-M+CMY+NDM genes. Conclusion- Experimental data suggest, albeit indirectly that TolC, porinYiaT may be playing a significant role along with NDM in mediating MDR mechanisms.

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03 GAURAV KUMAR

Development of Recombinant Yeast for Co-Fermentation of Pentose and Hexose Sugars.

Supervisors : Prof. R. V. Kuhad and Dr. Y. P. Khasa

Th 25322

Abstract
(Verified)

The climate changes, environmental pollution and the rapid depletion of fossil fuels demands for development of alternative strategies for fuel generation in sustainable manner. The use of fermentable sugars derived from lignocellulosics as a substrate for bioethanol production is an attractive approach. The major problem associated with bioethanol production from LCB is the inability to efficiently co-ferment both hexose and pentose sugars simultaneously. Industrially relevant yeast *Saccharomyces cerevisiae* lacks the ability to metabolise pentose sugars as it doesn't possess endogenous pathway for pentose metabolism. The naturally occurring pentose utilizing yeast cannot tolerate high ethanol concentration and are not found suitable for bioethanol production at industrial scale. Therefore, engineering of *S. cerevisiae* for enabling it to co-ferment both five and six carbon sugars present in hydrolysate is an attractive approach. In the current strategy, three different *S. cerevisiae* strains namely, HAU, BY4741, and CRY1 were engineered. The genes encoding for the oxidoreductive pathway (*xyl1, xyl2, xks1*), for isomerase pathway (*xyl a*) and xylose transporter (*aut1*) were introduced episomally in *S. cerevisiae.* Different strategies such as co-transformation, operon construction, and combinatorial regulon generation were designed where the enzymes involved in xylose catabolism were cloned in yeast shuttle vector. Among all the strategies, the combinatorial regulon method was found to be a promising approach where each gene of a combination was cloned as an individual regulon having its own promoter and terminator sequence. The developed transformant, namely *S. cerevisiae* HAU (*ura* -/-), harbouring $_{P}$G-XR-C$_{T}$-$_{P}$G-XDH-C$_{T}$ construct, exhibited maximum ethanol production of 4.59 g/L with a yield and productivity of $Y_{P/S}$ (g/g) 0.40 and 0.34 g/L/h, respectively. In sugarcane bagasse hydrolysate, the ethanol yield $Y_{P/S}$ (g/g) 0.40 and productivity of 2.24 g/L/h was obtained. The recombinant yeast thus generated was capable of co-fermenting glucose and xylose and yielded comparatively high ethanol titers.

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04 RAI (Gargi)

Determination of T Cell Subsets and their Effector Response to *Aspergillus* Antigen in Chronic Rhino (CRSwNP) and Toll Like Receptors mRNA Expression.

Supervisors : Dr. Shukla Das, Prof. V. G. Ramchandran, Dr. Sonal Sharma and Dr. Neelima Gupta

Th 25321

Abstract
(Not Verified)

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a persistent inflammatory condition affecting nasal sinuses. The exact pathogenesis remains unclear wherein various etiologies, like anatomical

variations, fungal infection as well as colonization, and atopic reaction with dysregulated immune response are some of the underlying factors involved. To play a critical role in CRSwNP, T cell subsets with Toll-like receptor (TLR)2 and TLR4 were recognized. However, its pathogenesis has been perplexed by conflicting reports on the role of Th17/Treg cells in patients of distinct ethnicities. In this study, the effect of *Aspergillus flavus* antigen on proportions of different T-lymphocyte subsets and cytokine secretion in peripheral blood mononuclear cells (PBMCs) of CRSwNP patients, before and after specific therapy and healthy controls, were studied. TLR2 and TLR4 expression analyzed in polyp tissue of cases and controls for disease co-relation. We noted increased levels of CD4+ CD45RO+ T cells and a marked immune imbalance, with elevated Th17 and decreased T regulatory in PBMCs of CRSwNP patients after *A. flavus* stimulation. Comparatively, Interleukin (IL)-17, IL-10 and IFN-γ levels were increased, with low IL-4 and TGF-β levels in PBMCs supernatant of patients after *A. flavus* stimulation. The mRNA expression of TLR2 was significantly upregulated in polyp tissue of CRSwNP cases as compared to controls. Our data highlights that the wide pool of memory (CD4+CD45RO+) T cells provides a potential reservoir of immediate response to the *A. flavus* stimulation. Excessive expression of TLR2 contributing to the imbalance in Th17/Tregs population in patients of CRSwNP. After therapy, recovery of Tregs cells showed a favourable phenotype result, although high circulating CD4+CD161+ may be harmful to patients predisposed to future recurrences. Constant exposure and tendency of *A. flavus* to colonize nasal cavities can lead to a Th17 driven inflammation. Th17 with TLR2 promotes resistance to treatment and progresses to chronicity.

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05 SAINI (Sanjesh)
Role of microRNA in Pathogenesis of Influenza A Virus Infection.
Supervisors : Prof. Malini Shariff and Prof. Madhu Khanna
Th 25319

Abstract
(Not Verified)

Influenza viruses are among the major pathogen of respiratory tract infection. Influenza viruses usually causes seasonal and endemic infection, but influenza virus always carries a risk of unpredictable pandemics. A viral pandemic could infect half of the population in a single pandemic year, which may drastically reduce the average life expectancy (1). The four major influenza pandemic happened in the last century, killed millions of people worldwide. The highly mutating nature of influenza virus helps the virus to evade host immune system and also contributes to drug resistance. A high degree of drug resistance has been reported among all the approved drugs against influenza. The drug resistance against earlier FDA approved anti-influenza drugs Amantadine and Rimantadine in circulating strain was 0.4% during 1994–1995, which increased to 2.3% during 2003–2004. During the year 2005–06, the drug resistance against these drugs observed 92%. Therefore these drugs are currently not recommended for treatment of influenza. This threatening evolution of influenza is the major reason that the influenza vaccine formulation is revised annually to accommodate the genetical changes arose in circulating strain. Therefore it is quite necessary to understand the host cellular pathways involved in virus pathogenesis to design better therapeutics alternative having a minimum effect on genetical variability.The current study involves assessment of microRNAs and unfolded protein (UPR) response in the pathogenesis of influenza A virus infection. The miR 141 and miR-155 are chosen for the study. The miR-141 and miR-155 observed significantly upregulated during influenza infection. Similarly, the UPR pathways also observed modulated by the influenza virus infection. The expression of ATF-6 and PERK pathways observed significantly down-regulated during influenza virus infection. We further analyzed the association of miR-155 and miR-141 with UPR response. It was observed that miR-155 significantly activates the IRE1

pathway of UPR as evident by an increase in splicing of XBP-1 upon miR-155 transfection. Since the UPR and miR-155 are associated in pathogenesis of various other diseases and pathogen born infections. The study may have a broad impact other than a better understanding of the influenza virus pathogenesis. The miR-155 also observed to modulate the inflammatory and antiviral response during influenza infection by negatively regulating the SOCS-1. The "cytokine storm" and excessive inflammatory response are known to modulate infection outcome.Therefore miR-155 regulation amy have significant in disease progression. In contrary to the miR-155, we have not observed any significant association of miR-141 with UPR and inflammatory response. But, miR-141 observed to modulates the expression of eif4E during influenza infection. The eif4E is an essential component for cap-dependent translation in eukaryotic cells. It was observed that influenza mediated miR-141 expression negatively regulate the eif4E and contribute to host protein shut off. Influenza mediated inhibition of eif4E was relieved by transfection of miR-141 inhibitory RNA, which suggest a significant role of miR-141 in influenza virus pathogenesis. The Nonstructural protein (NS1) is an important viral protein is known to act as a master regulator during influenza virus infection. The NS1 counters various host pathways for efficient viral replication. We also studied the co-rrelation between NS1 gene and microRNAs. But, our data suggest theat there is no significant co-relation exist between miR-141 or miR-155 expression and with NS1 gene. Further, we have analyzed the effect of miR-155 and miR-141 on the replication of influenza virus. We observed that the transfection of miR-155 mimic and miR-141 inhibitor significant repression in the replication of influenza virus.

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06 SAINI (Meenu)

Characterization of Gamma-GlutamylTranspeptidase from Bacillus atrophaeus GS-16: Application in Synthesis of γ–D-glutamyl-L-tryptophan an Immunomodulatory Peptide.

Supervisor : Prof. Rani Gupta

Th 25316

Abstract
(Not Verified)

Gamma-glutamyltranspeptidase (GGT; E.C.2.3.3.2) is a highly conserved and ubiquitous enzyme that acts by cleaving the γ-amide bond of donor substrates and transfers the γ-glutamyl moiety to another acceptor substrate. Microbial GGTs find application in synthesis of several γ-glutamyl peptides of commercial value. The present work focuses on characterization of GGT from *Bacillus atropaheus* GS-16 (BaGGT) and enzymatic synthesis of an immunomodulatory peptide γ-D-glutamyl-L-tryptophan (SCV-07). *B. atrophaeus* secreted GGT enzyme extracellularly which was optimized to 3.5 fold with 3710 ± 112 U/L production titres. The purified enzyme existed as heterooctamer (~240 kDa) under native conditions and showed two bands corresponding to large subunit (~45 kDa) and small subunit (~21 kDa) on SDS-PAGE. BaGGT was found to be optimally active at pH 10.0 and 50 °C and exhibited broad acceptor substrate specificity. The enzyme was heterologously expressed in *E. coli* and subsequently purified using Ni-NTA affinity chromatography. Biochemical properties of recombinant enzyme were similar to wild-type BaGGT enzyme. Molecular characterization of 14 amino acid-long extra sequence by site-directed mutagenesis revealed its role in folding, autoprocessing and overall conformation of the enzyme. Among 20 mutants generated, a mutant E374N showed 2 fold enhanced catalytic activity. Further protein engineering at position 459 in the neighbourhood of oxyanion hole resulted in development of a mutant A459I with improved transpeptidation and 3 fold increased catalytic efficiency. Finally, the best mutant A459I was immobilized onto Fe_3O_4-chitosan magnetic nanoparticles and used for SCV-07 synthesis. A conversion rate of ~70% corresponding to 35.2 ± 1.7 mM product yield was obtained with minimized byproduct. The immobilized enzyme was found to be

fully active till 6 consecutive cycles with similar product formation as analysed by HPLC. The SCV-07 product was purified by Dowex acetate form resin and confirmed by HPLC and proton NMR.

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07 YADAV (Aarti)
Functional Characterization of LeishmaniaDonovani Cdc45: Role of PIP Box.
Supervisor : Prof. Swati Saha
Th25317

Abstract
(Not Verified)

Eukaryotic DNA replication is a generally conserved process. Annotation of *Leishmania* whole genome sequences indicated the same, though some conserved proteins are absent. This work was undertaken to characterize the Cdc45 protein, a component of the replicative helicase. By creating genomic knockout lines and examining their phenotypes we established that Cdc45 is essential, with its depletion leading to growth and cell cycle defects, prolonged DNA replication, and poor survival in host macrophages. These defects were rescued by ectopic expression of Cdc45. LdCdc45 was found to carry a PCNA-interacting protein (PIP) box. PCNA is the clamp protein controlling processivity of the DNA polymerases, interacting with several replication proteins but with no reports of interaction with Cdc45. PCNA partners bind PCNA via a PIP box in most cases. On analyzing the structure of LdCdc45 against the human Cdc45 crystal structure the PIP motif was found opposite to the interface involved in interactions with Mcm2-7, thus predicted to be available for interaction with PCNA. Using wild type and Cdc45-PIP mutant, we found PCNA and Cdc45 interacted stably in cell extracts, the two proteins interacted directly, and the interaction was mediated via the PIP box. Analyses of chromatin-bound protein fractions of *cdc45* knockout cells ectopically expressing either wild type Cdc45 or Cdc45-PIP mutant protein suggested that the interaction may help recruit or stabilize the PCNA-polymerase complex at the replication fork. Cdc45 PIP box was essential for *Leishmania* cell survival. Complementation assays revealed the *S. pombe* Cdc45 PIP box was also essential for cell survival. Separate investigations will ascertain if Cdc45 and PCNA interact in other eukaryotes. The findings of this thesis underscore the fact that there are diverse facets in modes of DNA replication among eukaryotes even though the process is broadly conserved, also indicating the relevance of studying this process in non-conventional eukaryotes.

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