CHAPTER 36

MICROBIOLOGY

Doctoral Theses

01. ADIVITIYA
High Level Production of Therapeutically Important Recomninant Streptokinase in Pichia Pastoris : Process Optimization and Biophysical Characterization.
Supervisor: Dr. Y. P. Khasa
Th24258

Abstract
(Verified)

Streptokinase is the most widely used thrombolytic agent in the developing countries due to its cost-effectiveness. Many upstream bottlenecks such as protein toxicity and plasmid instability affect its over-expression in prokaryotic hosts like E. coli while a uniformly poor expression yield was reported in eukaryotic hosts. Therefore, in the current study, the streptokinase gene from Streptococcus dysgalactaie subsp. equisimilis strain H46A was cloned and expressed in P. pastoris to achieve its high level expression. Optimization of host-vector combinations helped in production of 582 mg/L rSK and 538 mg/L rSK-His upon induction at OD600 20. Screening of two copy clones improved production to 758 mg/L and 774 mg/L of rSK-His and rSK respectively. The identity of the full length and C-terminally degraded streptokinase fragments was confirmed by MALDI-TOF/TOF spectroscopy. Purified rSK-His and rSK showed a specific activity of 64,903 IU/mg and 55,240 IU/mg respectively. The protein showed 15.43% α-helix and 26.43% β-sheet with a fluorescence emission maxima at ~347 nm. Deglycosylation at residue 14 resulted in reduced molecular weight and secretion yield as well as an increase in its proteolytic degradation. Mutation of K59 residue helped in improving the resistance of streptokinase towards plasmin-mediated degradation. Increasing the gene dosage to four copies under the PIR1 gene's signal sequence improved rSK-His(PIR1) titre to 1206.48 mg/L with a YP/X of 161.87 mg/g DCW. The cell retention strategy, at shake flask, resulted in 3049.53 mg/L rSK-His(PIR1) with a YP/X of 167.65 mg/g DCW and improved product quality. A streptokinase molecule with an authentic N-terminus (N-rSK) was produced up to 1062 mg/L with a specific activity of 67,552.61 IU/mg. Upon bioreactor cultivation, soya flour hydrolysate and urea supplementation during induction phase along with stringent control over cultivation parameters ameliorated overall product quality and yield to 4.03 g/L with a high volumetric productivity of 52.33 mg/L/h.

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02. GAUR (Nivedita)

The Role of Human Associated Herpesvirus Latent Antigens in Epithelial to Mesenchymal Transition of Cancer Cells.

Supervisor : Dr. Rajeev Kaul

Th 24125

Abstract
(Verified)

Cancer is a major concern as it accounts for millions of deaths every year and 15-20% of these cases are caused by human tumor associated-virus. Herpesviruses figure among few of the most important cancer causing infectious agents. EBV and KSHV are both γ-herpesviruses and are associated with many human associated malignancies of both epithelial and lymphoid origin. Both the viruses establishes asymptomatic persistent infections otherwise known as latent infections. To establish latent infections virus encodes for latency associated protein. EBV expresses eleven latency associated viral proteins including EBV nuclear antigen1 (EBNA1) and EBNA3C. KSHV also encodes latency associated proteins including LANA. In our present study we investigated role of specific viral latent proteins coded by EBV (EBNA1, EBNA3C) and KSHV (LANA) in promotion of epithelial to mesenchymal transition (EMT). It is the initial step in the process of metastasis and cancer progression. To investigate the role of selected viral latent proteins in EMT we investigated the effect of these latent proteins on expression of certain cellular genes that are critical for the process of EMT of cancer cells. These included Snail, Slug, β-catenin, TCF8/ZEB1, vimentin, E-cadherin, N-cadherin, ZO1 and claudin. Our results showed that expression of EBNA1, EBNA3C and LANA resulted in significant upregulation of transcriptional factors like Snail, Slug, β-catenin, TCF8/ZEB1 and vimentin signifying mesenchymal transition of cancer cell and downregulation of membrane proteins like E-cadherin, ZO-1 and claudin which results loss of cell-cell adhesion and increased migration. The changes we observed in the expression, organization and localization of EMT associated cellular proteins in cells expressing viral latency associated proteins clearly indicated critical role of viral antigens in the process of EMT. The study also suggests that these latent antigens can promote migration and invasion of cancer cells via promoting the process of EMT.

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03. PAUL (Catherine Skyougzin)

Role of Hepatitis C Virus (HCV) Core protein in Virus Mediated Tumorigenesis by Modulation of Cellular Metastasis Suppressor Nm23-H1.

Supervisor : Dr. Rajeev Kaul

Th 24124

Abstract
(Verified)

Hepatitis C Virus (HCV) is the major cause of hepatocellular carcinoma (HCC) which is the most prevalent liver cancer throughout the world. The primary mode of transmission of the virus is by parenteral exposure via blood. HCV belongs to the *Flaviviridae*family and *Hepacivirus*genus and is a cytoplasmic replicating virus. In contrast to HCC caused by Hepatitis B Virus (HBV), HCV causes HCC with increased incidence of metastasis which leads to poor prognosis of HCV associated HCC. Metastasis is the transfer of tumor from its primary origin to different parts/organs of the body. The onset of HCC is assumed to be triggered via indirect effect of immune mediated chronic inflammation. However HCV can

cause HCC by affecting various host pathways that are involved in proliferation, angiogenesis, epithelial-mesenchymal transition (EMT), DNA repair, apoptosis and oxidative stress. Several HCV coded proteins are known to induce pro-metastasis cellular functions. One of the best studied cellular metastasis suppressor protein which is known to be modulated in various viral cancers is Nm23-H1. Our study now shows that HCV-Core expression as well as HCV infection is pro-metastatic which is coincidental with Nm23-H1 transcriptional downregulation and protein degradation. Also, HCVCore co-localizes with Nm23-H1 within the cytoplasm of the cells in both HCV-Core overexpressed as well as virus infected cells. Further, HCV-Core and Nm23-H1 are found to interact stably with each other. The domains of Nm23-H1 and HCV-Core essential for this interaction have been identified which were found to be amino acid region from 95-114 and 81-117 of Nm23-H1 and HCV-Core respectively. Core expression can also rescue the cells from metastasis suppression effect of Nm23-H1. Our study provides the role of HCV-Core in HCV mediated cancer metastasis.

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04. SINGH (Nambram Somendro)

Detection and Analysis of Genes Conferring Resistance to β-Lactam and Quinolone Antibiotics, and their Genetic Environment in Escherichia Coli Strains Isolated from an Urban Aquatic Environment

Supervisor : Prof. J. S. Virdi

Th 24126

Abstract
(Verified)

A high prevalence of resistance to β-lactam, quinolone and other antibiotics in *Escherichia coli* inhabiting the aquatic environments is a world-wide public health concern. The situation is further complicated as the genes conferring resistance to these antibiotics can be easily transferred among bacterial species with the help of mobile genetic elements - plasmids, integrons, insertion sequences (IS) and transposons. The analysis of genetic environments and mobile genetic elements associated with antibiotic resistance genes is therefore important because, it provides useful information about the molecular epidemiology of these genes and their spread. In the present study the genetic environment of β-lactamases genes (*bla*, *bla*, and *bla*), and the plasmid-mediated quinolone resistance (PMQR) genes (*qnrA - qnrD*, *aac(6')-Ib, qepA*, *oqxA*, and *oqxB*) in 61 *E.coli* strains isolated from the river Yamuna traversing the National Capital Territory of Delhi (India) were studied. The structure of class 1 integrons and their gene cassettes were also analysed. The study revealed that insertion sequence IS*26* was present upstream of *bla*; insertion sequence IS*Ecp1* was present upstream of *bla*and the *orf477* was present downstream of *bla*. IS*Ecp1* was also present upstream of *bla*and, *blc*and *sugE*were present in the downstream region. IS*Ecl2* was present upstream of the *qnrS*gene. In conclusion, the overall genetic environments surrounding these genes in *E.coli* isolated from India were similar to those reported from *E.coli* strains isolated globally. Conjugation assays, and analysis of the plasmid DNA of the transconjugants indicated that β-lactamases genes, the PMQR genes, and class 1 integrons were plasmid-mediated and possibly transmitted between species through horizontal gene transfer. This might lead to dissemination of antimicrobial resistance genes in the aquatic environments. This is also the first report describing the genetic environments of *bla*genes, PMQR genes and integrons in aquatic *E.coli* isolated from India.

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05. UDITA

The Functional Role of Histone Acetyltransferase HAT2 in Leishmania Donovani.

Supervisors : Prof. Swati Shah

Th 24128

Abstract
(Verified)

This study investigates the role of histone acetyltransferase HAT2 in Leishmania donovani. We found HAT2 to be constitutively nuclear and to acetylate histone H4 at K10 residue both in vitro and in vivo. Depletion of HAT2 led to aberrant growth, cell cycle and DNA replication patterns. Cell cycle defects were caused by downregulation of cyclins CYC4 and CYC9. Ectopic expression of CYC4 and CYC9 in HAT2-depleted cells rescued these cell cycle defects. Genes in the Leishmania genome are organized such that functionally unrelated genes are clustered together and co-ordinately transcribed polycistronically and constitutively. By chromatin immunoprecipitations using H4acetylK10 antibodies we found the sites of these transcription initiations to be enriched in H4K10 acetylation throughout the cell cycle, though to a lesser extent in HAT2-depleted cells, supporting a role for this acetylation mark in regulation of transcription. However, microarray analyses revealed that the genes associated with CYC4 and CYC9 in their clusters are not co-ordinately downregulated in HAT2-depleted cells. Reporter assays revealed the presence of internal promoters in the clusters, in the regions immediately upstream of CYC4 and CYC9. Chromatin immunoprecipitation assays uncovered H4acetylK10 enrichment at these internal promoters only at those cell cycle stages where the cyclins were needed, and real time PCR analyses revealed that this was coupled to upregulation of expression of these genes. While downregulation of H4K10 acetylation in HAT2-depleted cells was detected at both the primary transcription initiation sites as well as the gene-specific promoters, the latter were more sensitive to H4K10 acetylation levels and therefore led to downregulation of those particular genes. Our findings underline the complexity of transcriptional regulation in trypanosomatids.

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