CHAPTER 40

MICROBIOLOGY

Doctoral Theses

427. ARORA (Jasmine)
Characterization of the DNA Replication Protein Orc1/Cdc6 in the Archaeon Picrophilus Torridus.
Supervisor : Prof. Swati Saha
Th 22646

Abstract

Eukaryotic DNA replication is preceded by the assembly of prereplication complexes (pre-RCs) at or very near origins in G_1 phase, which licenses origin firing in S phase. The archaeal DNA replication machinery broadly resembles the eukaryal apparatus, though simpler in form. The eukaryotic replication initiator origin recognition complex (ORC), which serially recruits Cdc6 and other pre-RC proteins, comprises six components, Orc1-6. In archaea, a single gene encodes a protein similar to both the eukaryotic Cdc6 and the Orc1 subunit of the eukaryotic ORC, with most archaea possessing one to three Orc1/Cdc6 orthologs. Genome sequence analysis of the extreme acidophile Picrophilus torridus revealed a single Orc1/Cdc6 (PtOrc1/Cdc6). Biochemical analyses show MBP-tagged PtOrc1/Cdc6 to preferentially bind ORB (origin recognition box) sequences. The protein hydrolyzes ATP in a DNA-independent manner, though DNA inhibits MBP-PtOrc1/Cdc6-mediated ATP hydrolysis. PtOrc1/Cdc6 exists in stable complex with PCNA in Picrophilus extracts, and MBP-PtOrc1/Cdc6 interacts directly with PCNA through a PIP box near its C terminus. Furthermore, PCNA stimulates MBP-PtOrc1/Cdc6-mediated ATP hydrolysis in a DNA-dependent manner. This is the first study reporting a direct interaction between Orc1/Cdc6 and PCNA in archaea. The bacterial initiator DnaA is converted from an active to an inactive form by ATP hydrolysis, a process greatly facilitated by the bacterial ortholog of PCNA, the β subunit of Pol III. The stimulation of PtOrc1/Cdc6-mediated ATP hydrolysis by PCNA and the conservation of PCNA-interacting protein motifs in several archaeal PCNAs suggest the possibility of a similar mechanism of regulation existing in archaea. This mechanism may involve other yet to be identified archaeal proteins.

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428. ARTI KUMARI

Biochemical and Molecular Characterization of Enantioselective Lipases from Trichosporon asahii MSR54: Their Role in Biofilm Formation.

Supervisor : Prof. Rani Gupta

Th 22273

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429. BAJAJ (Priyanka)

Understanding Clonal Diversity and Molecular Mechanisms of β-lactamase Mediated Antibiotic Resistance Among Escherichia Coli Strains Isolated from an Indian Urban Aquatic Environment.

Supervisor : Prof. J. S. Virdi

Th 22272

Abstract

Escherichia coli strains (n=126) were isolated from water samples collected along the entire stretch of the river Yamuna traversing National Capital Territory of Delhi. These were presumptively identified using API 20E strips, and differentiated as sorbitol-fermenting (SF; 90 %) and non sorbitol-fermenting (NSF; only 10%). The strains belonged to phylogroup A (59%), B1 (25%), B2 (1%), and D (15%). Of these, a collection of 61 strains representing all phylogroups was studied further. These were confirmed by sequencing of 16S ribosomal RNA genes, and serotyped. Based on rep-PCR analysis, the NSF E. coli comprised a distinct genotype. Antimicrobial susceptibility revealed that 28% of E. coli strains isolated from Indian urban aquatic environment were resistant to ≥3 β-lactams. Resistance due to extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases was detected in 16% and 3% of the strains respectively due to the presence of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CMY\text{-}42}$ genes, in that order. ESBL and AmpC phenotype was most prevalent in phylogroup D which commonly harbors extra-intestinal pathogens. In silico analysis of plasmid-mediated AmpC β-lactamase families provided insights into their active site structures vis-à-vis phylogenetic relatedness, and suggested the most suitable inhibitor choices. Furthermore, high resistance to quinolones was observed in >50% strains which also showed ESBL or AmpC production. Resistance was primarily attributed to amino acid substitutions in the quinolone resistance-determining regions of GyrA (S83L +/- D87N), and ParC (S80I +/- E84K). The phenotypically susceptible strains nevertheless, harbored self-transmissible plasmid-mediated qnrS gene. The study describes the phylogenetic diversity of waterborne E. coli from Indian. It represents the first report of distribution and molecular characterization of genes encoding CTX-M and AmpC β-lactamases in E. coli isolated from an Indian urban aquatic environment. This also represents first report of quinolone co-resistance in such E. coli from India and brings forth their public health implications.

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430. CHAUHAN (Mayank Singh)
Identification of Dengue Virus NS5 Protein Region Responsible for Interleukin-8 Transcription and Secretion.
Supervisors : Dr. Anita Chakravari, Dr. P. Bhalla and
Dr. N. P. Singh
Th 22645

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431. GANDHI (Jaya)
Mechanism of Epstein-Barr Virus and Kaposi's Sarcoma-Associated Herpesvirus Lytic Regulation By Modulator of Inflammation Cyclo-Oxygenase-2.
Supervisor : Dr. Rajeev Kaul
Th 22647

Abstract

Subsequent to primary infection γ-herpesviruses follow two distinct life cycles in human host, a latent form of infection that allows the virus to persist in a dormant state for life time in host and a lytic form that produces new infectious virions. Understanding the switch between latency and lytic replication is an important problem in herpes virology. Lytic reactivation is a critical step in virus life cycle and is important for virus dissemination to new hosts and infection of new cells. One of the major enzymes to be closely associated and expressed during γ-herpesvirus directed malignancies is Cyclo-oxygenase-2 (COX-2). It has been earlier reported that patients suffering from chronic inflammatory conditions characterized by up-regulated COX-2 levels have high incidences of EBV associated malignancy. COX-2 is a key mediator of inflammatory pathways and its elevated expression has been found in several human cancers. COX-2 is known to play an important role in de novo infection and in maintenance of latent infection. In present study, we have shown the critical role of COX-2 enzyme in transition of both viruses from latency to lytic reactivation in latently infected cells. Further, the upregulated levels of COX-2 are coincident to the expression of EBV and KSHV lytic switch genes. Present study showed that COX-2 mediated lytic reactivation of EBV and KSHV occur via its downstream effector PGE2 which act through EP1 and EP2 receptors in both autocrine & paracrine mode. Also, the lytic reactivation mediated by over-expression of COX-2 generates intact and biologically infectious progeny virions. In summary, modulator of inflammation COX-2 has a direct role in regulation of γ-herpesvirus lytic reactivation and progression of EBV and KSHV mediated tumorigenesis. Thus, our

studies and observations add new horizon towards deeper understanding of the role of inflammation in the progression of cancers mediated by oncogenic γ-herpesvirus.

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432. JAIN (Kavish Kumar)
Enhanced Cellulase Production from a Thermophile Mould Thermoascus Aurantiacus RCKK and its Applications.
Supervisor : Prof. R. C. Kuhad
Th 22643

Abstract

Thermophilic fungi are potential source of thermostable enzymes and other value added products. Thermostable cellulases offer several advantages like higher rates of substrate hydrolysis, lowered risk of contamination and increased flexibility with respect to process design. The production of cellulases from a thermophilic fungus identified as Thermoascus aurantiacus RCKK (Acc. No. JN676149) has been optimized up to tray level under SSF with 2.5 fold than unoptimised. Crude enzymes were stable up to 70°C for more than 4 h. The CMCase enzyme of 35 kDa was purified to homogeneity and found to be thermostable ($t_{1/2}$ at 60°C -400 min, 70°C- 238 min, 80°C- 128 min) and pH stable. The efficiency for cellulose hydrolysis of enzyme was evaluated on diverse cellulosic substrates i.e. crystalline substrate avicel, wheat straw, office paper waste and algal pulp. The crude Cellulase was also tested for its pulp biorefining capability. In addition to hydrolases, T. aurantiacus RCKK was found to produce antioxidants as fermentation byproducts with significant %DPPH• scavenging, ferric reducing antioxidant property and in vivo antioxidant capacity against H_2O_2 treated Saccharomyces cerevisiae (28% survival) and H_2O_2 treated CHOK cell lines (up to 80%). Further, in order to increase production level of cellulases and eventually economize the process, heterologous expression of Endoglucasnase and β-glucosidase gene from T. aurantiacus RCKK in Pichia pastoris was carried out. Capability of T. aurantiacus RCKK to produce thermostable cellulases and antioxidants in a single process holds potential of commercialization.

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Supervisor : Prof. T. Satyanarayana
Th 22274

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Supervisor : Prof. R. K. Saxena
Th 22644

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Supervisors : Dr. Beena Uppal and Dr. Rakesh Singh
Th 22667

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436. KATHURIA (Shallu)
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Supervisors : Dr. Anuradha Chowdhary and Prof. Harbans S. Randhawa
Th 22275

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437. NISHA M
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Supervisor : Prof. T. Satyanarayana
Th 22276

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438. SINGH (Jyotsna)
Genetic and Functional Characterization of HIV-1 Rev and LTR Gene.
Supervisors : Prof. V. G. Ramachandran and Dr. A. C. Banerjea
Th 22642

Abstract

Although the HIV-1 epidemic in India is mainly due to subtype C, other subtypes have also been reported from different parts of India. Genetic analysis of HIV-1 Rev sample study suggested that there are very less sequence variation in samples collected from North India and most of the sequences resemble subtype C consensus sequence reported previously from India. HIV-1 LTR sequence analysis showed that four out of six samples formed a unique phylogenetic cluster which was close to subtype B. The transcription binding sites and regulatory regions showed some unique mutations which may potentially alter basal as well as Tat- mediated HIV-1 LTR promoter activation. Genetic analysis of HIV-1 Nef natural variants revealed some interesting mutations. The samples depending on relative resemblance to prototype Nef from HIV sequence database were divided into group B variants and B/C recombinants. Group B variants displayed higher degree of variations in all the functional domains with some novel mutations as compared to B/C recombinants. Functionally, HIV-1 Nef variants were found to have varying effects in their CD4, MHC and p21 downregulation activity. Genetic analysis of HIV-1 Vpu gene from twenty HIV-1 patients from North India has shown notable variations in five Vpu variants. Most of the mutations in Vpu were found in cytoplasmic domain while transmembrane domain is almost conserved in all the sequences. Bootscan and phylogenetic analysis of Vpu sequences revealed that all the variants resembled to subtype C. One remarkably noteworthy observation was the point mutation of phosphorylable serine residue ser52 and ser56 at beta-trcp binding motif in cytoplasmic domain of Vpu. During viral life cycle, CD4 degradation is very critical for optimum production of infectious virus particles. Thus, these variations including inter-subtype recombination occurring naturally in HIV-1 proteins have a strong impact on the pathogenesis of virus.

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