CHAPTER 36

MICROBIOLOGY

Doctoral Theses

01. ANUPAM PRAKASH
Study of Cryptococcus Species in Immunocompromised Patients.
Supervisors: Prof. Anuradha Chowdhary and Prof. H. S. Randhawa
Th 22784

Contents

1. Cryptococcus species and cryptococcosis: An overview 2. A survey of Cryptococcus species and cryptococcosis in HIV/AIDS and other immunocompromised patients in Delhi/New Delhi hospitals 3. Environmental distribution of various Cryptococcus species and some other yeast-like fungi in climatically divergent regions of India 4. In vitro antifungal susceptibility profiles of Cryptococcus species isolated from clinical and environmental sources 5. Multilocus sequence typing (MLST) of Cryptococcus neoformans var. grubii and C. gattii isolated from clinical and environmental sources. Overall summary and conclusions. Appendices. List of publications.

02. BOSE (Himadri)
Production, Characteristics and Applications of γ-Carbonic Anhydrase of the Polyextremophilic Bacterium Aeribacillus Pallidus TSHB1.
Supervisors: Prof. Rani Gupta and Prof. T. Satyanarayana
Th 23187

Abstract
(Not Verified)

The main contributor of global warming is carbon dioxide emitted from industries which is resulting in many climate related hazards. Mineralization of carbon dioxide by employing biocatalyst Carbonic Anhydrase (CA) using divalent metal ions offers a cost effective and environmentally safe solution for long term storage of carbon dioxide. Prokaryotes found in extreme environments can be a source of thermostable and alkali-stable CAs. A moderately thermophilic, alkaliphilic and halotolerant bacterium Aeribacillus pallidus TSHB1 was isolated from Choti Anhoni hot spring, Madhya Pradesh, India which produced high titres of carbonic anhydrase. Enzyme production was optimized by OVAT and statistical approaches which led to 3.76-fold enhancement in thermo –alkali stable CA production (4700 U $gdbm^{-1}$). Molecular mass of the purified protein determined by SDS PAGE is 32 ± 2 kDa and native mol. wt. is 96 kDa, therefore, the enzyme is a homotrimer, hence a γ-CA .The enzyme is alkalistable, and moderately thermostable The enzyme is halotolerant and stable in the presence of flue gas contaminants like SO_3^{2-} ,NO_3^{2-} and SO_4^{2-} but strongly inhibited by heavy metals like Pb^{2+}, Hg^{2+}. Hence CA from A.pallidus TSHB1 is a suitable candidate for capturing CO_2 from flue gas. The enzyme was successfully used in mineralizing CO_2 present in motorbike exhaust containing about 14% CO_2. CA produced calcite form of calcium carbonate. Upon substitution of active site Zn^{2+} with Mn^{2+}, the enzyme could function as a peroxidase. The enzyme was successfully immobilized on silanized magnetic iron oxide nanoparticles. β – CA (CA193) and γ – CA (γCA176) encoding genes from A. pallidus have been cloned in E. coli but the genes were expressed in an inactive form. The recombinant proteins could not be activated despite several attempts. The in-silico analysis of both CA encoding genes was performed using various bioinfromatic tools.

Contents

1. Introduction. 2. Materials and methods 3. Results 4. Discussion 5. Summary and conclusions. References. Publications, workshops and presentations.

03. GOYAL (Ritu)
Pathogenecity Testing and Molecular Typing of Candida Species Isolated from HIV/AIDS Patients.
Supervisors: Dr. Ravinder Kaur and Dr. Preena Bhalla
Th 22785

Contents

1. Introduction. 2. Lacunae in existing knowledge 3. Objectives 4. Review of literature 5. Materials and methods 6. Results and observations 7. Discussion 8. Summary 9. Conclusion 10. Bibliography 11. Appendix-I: Buffers/reagent 12. Appendix-II: Clinical proforma, consent form 13. Appendix-III: Abbrevations, master chart 14. Appendix-IV: Publications.

04. MALIK (Monika)
Consequences of Autoantigenicity of Apoptotic Blebs in Systemic Lupus Erythematosus.
Supervisors: Dr. V. G. Ramachandran and Dr. Rahul Pal
Th 22786

Contents

1. Introduction. 2. Review of Literature 3. Materials and methods 4. Results 5. Discussion 6. Summary and conclusions. Bibliography. Appendix. Publication.

05. MD ANZAR ASHRAF
Mutational Analysis of IRES, E2 and NS5A Region of HCV and their Relationship to Pegylated Interferon Ribavirin Therapy Responses.
Supervisors: Dr. Anita Chakravarti and Dr. Premashish Kar
Th 23188

Contents

1. Introduction 2. Lacunae 3. Objectives 4. Review of Literature 5. Material and methods 6. Results 7. Discussion 8. Summary 9. Conclusions 10. Bibliography 11. Chemicals and buffers, consent form, proforma of patient, master chart 12. Publications.

06. NARESH KUMAR
Expression Analysis of an Array of Genes of Mycobacterium tuberculosis Clinical Isolates from Pulmonary Tuberculosis and Lymph Node Tuberculosis: Search for Mycobacterial Factors Associated with Differential Clinical Manifestation .
Supervisors: Prof. Mandira Varma-Basil and Prof. Mridula Bose
Th 223076

Contents

1. Introduction. 2. Review of literature 3. Collection of M. tuberculosis form lymph node tuberculosis (LNTB) and pulmonary tuberculosis (PTB) patients and culture in

7H9 media 4. To study the changes in lipid profile of clinical isolates of MTB from LNTB and PTB patients grown under different culture conditions 5. Expression profile of genes involved in lipid metabolism in MTB from LNTB and PTB clinical isolates under different culture conditions as mentioned above 6. Expression profile of the identified genes in THP-1 cells infected with mycobacteria. Summary and conclusions. References. Appendix. List of Publications.

07. NIRMALA DEVI

Bioprocess Optimization of Recombinant Human Interleukin-7 (hIL-7) in Escherichia Coli and Methylotrophic Yeast Pichia Pastoris.

Supervisor: Prof. Dr. Yogender Pal Khasa

Th 23077

Abstract
(Not Verified)

hIL-7 gene was cloned into various expression vectors of E. coli under strong T7 promoter with and without 6x-His tag, where 80 mg/L of hIL-7 protein was found in the form of IBs. The N-terminus fusion of small ubiquitin-like modifier (SUMO), thioredoxin (Trx) and N-utilization substance (NusA) tags increased the expression in the range of 90-140 mg/L where > 90% of the fusion protein was obtained in soluble form. A preparative scale affinity chromatography resulted in a high recovery yield of 50.6 mg/L with ~90% purity. The bioreactor studies were optimized with 6xHis-tagged, nontagged hIL-7 and SUMO-hIL-7 protein at fermentor level. After optimization of bioreactor studies, the highest hIL-7 protein expression was at a level of 3.28 g/L, 1.46 g/L and 2.65 g/L which showed approximately forty fold higher production in comparison to literature reports. A preparative scale affinity chromatography resulted in a high recovery yield of 50.6 mg/L with ~90% purity. The biological activity of purified/or refolded hIL-7 protein was measured by its ability to proliferate the 2E8 cell line, where it showed comparable proliferation efficacy with hIL-7 standard. The P. pastoris expression system is commonly used for the expression of the recombinant protein of eukaryotic origin. The fusion tags such as GFP and hSUMO had no effect on protein solubility which was produced in form of IBs. The recombinant P. pastoris GS115 PDI strain having protein disulfide isomerase was also tested for the expression of the fusion protein but failed to enhance the solubility of hSUMO-hIL-7 protein. Different signal sequences i.e. α-mating factor, PHO1, Pir1 and Pir2 were tested for the secretion of hIL-7 but failed to export hSUMO-hIL-7 protein in culture supernatant. The IBs of hSUMO-hIL-7 protein was isolated from P. pastoris cell pellet, refolded and tested for its biological activity using 2E8 cell line.

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08. PARASHAR (Deepak)

Bioprocess Development and Applications of the Recombinant Acid Stable α-Amylase from the Bacterium Bacillus Acidicola Tsas 1.

Supervisor: Prof. T Satyanarayana

Th 22787

Abstract
(Verified)

The recombinant acidstable α-amylase of acidophilic bacterium Bacillus acidicola TSAS1 has been produced extracellularly using a combination of cloning (E. coli and P. pastoris) and physico-chemical treatment strategies. Ba-amy expressed under GAP and AOX promoters in P. pastoris was comparable (150, 000 UL-1), which is 15-fold higher than that of the wild strain. The pure Ba-amy expressed in P.

pastoris is a glycoprotein of 66 kDa, which is optimally active at pH 4.0 and 60 °C with a T1/2 of 25 min at 70 °C. Furthermore, the Ba-amy was fused with DNA fragments encoding partial N- and C-terminal domains of thermostable α-amylase gene (Gt-amy) of Geobacillus thermoleovorans. The chimera (Ba-Gt-amy) expressed in E. coli displayed marked increase in catalytic efficiency and higher thermostability than Ba-amy.A high extracellular titre of Ba-Gt-amy (750 U mL-1) was attained by cloning it in P. pastoris and screening of a multi copy integrated clone (Amy-AOX3) through multiple selection markers. In order to saccharify starch in a single step, a chimera was constructed by using the Ba-Gt-amy and glucoamylase from Aspergillus niger. Both enzymes were joined together by using a linker peptide of 26 amino acids. Glucoamylase was of 75 kDa, while Amy-Glu was approximately of 145 kDa. Amy-Glu and glucoamylase show similar pH profile with good activity in acidic condition as that of Ba-Gt-amy with optimum at 4.0 – 5.0. The hydrolysis of raw starches revealed that Amy-Glu saccharifies wheat and corn starch efficiently as compared to the Ba-Gt-amy and glucoamylase with equimolar amounts of protein.

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09. SANDEEP KUMAR
Cloning, Expression and Application of Bacterial Pectinase and Laccase.
Supervisor: Prof. R. C. Kuhad
Th 23189

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10. SHARMA (Shweta)
Study on Expression Profile of microRNAs in Human Papillomavirus Mediated Cervical Carcinogenesis.
Supervisors: Prof. Shukla Das, Dr. Mausumi Bharadwaj, Prof. V .G. Ramachandran and Dr. Sonal Sharma
Th 22789

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11. SHAZIA FARIDI
Characteristics and Applications of the Native and Recombinant α-carbonic Anhydrase of the Polyextremophilic Bacterium Bacillus Halodurans TSLVI
Supervisor: Prof. T Satyanarayana and Prof. Rani Gupta
Th 22788

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12. SINGH (Pooja)
Utilization of Cholesterol by mce4A (Rv3499c) Overexpressed and mce1A (Rv0169) Overexpressed M. tuberculosis H37Rv and the Effect of Calcium Channel Blocker.
Supervisor: Prof. Mandira Varma-Basil and Prof. Mridula Bose
Th 22790

Contents

1. Introduction 2. Review of literature 3. To study the difference in the growth kinetics of recombinant M. tuberculosis H37Rv i.e. mce4A overexpressed (M. tb:mce4A↑) and mce1A overexpressed (M. tb:mce1A↑) grown in presence of cholesterol as sole carbon source 4. To study the changes in expression of genes involved in propionate catabolic pathway in recombinant strains of M. tuberculosis and wild type H37Rv grown in the presence of cholesterol and propionate catabolic pathways inhibitors 5. To study the effect of inhibition of propionate catabolic pathway on apolar and polar lipids of recombinant (mce4A overexpressed and mce1A overexpressed) and wild type H37Rv using cholesterol as a sole carbon source 6. To quantify cholesterol level in macrophages infected with recombinant M. tuberculosis at varying calcium concentrations and to study the effect of calcium channel blocker on the growth of recombinant M. tuberculosis inside macrophages. Summary and conclusions. References. Appendix. List of publications.

13. VIKAS KUMAR
Bioprecss Optimization of Recombinant Human Interleukin-3 (hIL-3) in Escherichia coli and Pichia Pastoris.
Supervisor: Prof. Dr. Y. P. Khasa
Th 22791

Abstract
(Verified)

Human interleukin-3 (hIL-3) is a pleiotropic cytokine used as a therapeutic agent in various clinical setting such as immunodeficiency disorders, hematological malignancies, and cytopenias. However, earlier reports have indicated a poor expression of the hIL-3 protein in the range of 10-100 mg/L from various heterologous hosts such as E. coli, B. subtilis, S. lividans, P. pastoris and mammalian cells. The codon-optimized hIL-3 gene was cloned into various expression vectors under strong T7 promoter with and without 6x-His tag, where 225 mg/L of hIL-3 protein was found in the form of inclusion bodies (IBs). The hIL-3 gene was also cloned with different signal sequences (pelB, asp, ompA, osmY and YebF) and cytoplasmic solubility enhancer tags (TrxA, GST, and SUMO), where protein expression was observed in the form of IBs. The IBs were solubilized, refolded and purified upto 95% purity with 53% recovery yield. The fed-batch cultivation optimization resulted in a protein concentration of 3.36 g/L at bioreactor level. The P. pastoris expression system is commonly used for the expression of the recombinant protein of eukaryotic origin to have PTMs. The expression of the hIL-3 protein was maximum in the case of 8-copies clone where in-vitro multimerization was used. The batch and fed-batch cultivation in complex medium resulted in hIL-3 protein concentration of 1.81 and 1.49 g/L, respectively. The batch and fed-batch cultivation in basal salt medium resulted in 2.23, and 1.45 g/L of hIL-3 protein, respectively. The role of N-glycosylation on its secretion, stability and biological activity were also studied. hIL-3 protein was purified by affinity chromatography and further deglycosylated using PNGase F enzyme. Conformational studies were done by CD and fluorescence spectroscopy. The biological activity of purified/or refolded hIL-3 protein was measured by its ability to proliferate the TF-1 cell line, where it showed better proliferation efficacy than hIL-3 standard.

Contents

1. Introduction and objectives 2. Review of literature 3. Materials and methods 4. Expression of hIL-3 in E. coli 5. Expression studies in P. pastoris 6. Summary and conclusion 7. Bibliography.