

CHAPTER 4

BIOCHEMISTRY

Doctoral Theses

01. BHATT (Shruti)
Pathophysiological Investigations into Sickle Cell Disease and Interventions to Improve Associated Hemorheological Abnormalities.
Supervisors: Prof. Alo Nag and Prof. Suman Kundu
Th 27349

Abstract

This thesis delves into the complex pathophysiological mechanisms underlying Sickle Cell Disease (SCD), focusing on the polymerization of hemoglobin S (HbS) and its consequences on red blood cell (RBC) morphology and function. The aberrant polymerization of HbS leads to the formation of rigid, sickle-shaped RBCs, impairing their ability to traverse microvasculature and deliver oxygen to tissues. This structural alteration initiates a cascade of adverse effects, including hemolytic anemia, vaso-occlusive crises, and chronic inflammation. To address these challenges, the research adopts a multifaceted approach. Firstly, it investigates the metabolic and elemental profiles in SCD patients using advanced multi-omics techniques such as liquid chromatography and inductively coupled plasma mass spectrometry (LC-MS/ICP-MS). By analyzing these profiles, the study aims to uncover novel biomarkers and therapeutic targets for SCD management. Secondly, the study evaluates the ex-vivo antisickling efficacy and toxicity of Sailin-HbS, an herbal formulation derived from traditional Ayurvedic knowledge. Sailin-HbS is designed to inhibit HbS polymerization and mitigate RBC biomechanical breakdown in SCD. Through rigorous experimentation, including ex-vivo assays and toxicity studies in animal models, the research seeks to assess the therapeutic potential and safety profile of Sailin-HbS, laying the groundwork for potential clinical application. Moving beyond preclinical assessment, the thesis progresses to clinical evaluation, focusing on the safety, tolerability, and efficacy of Sailin-HbS in improving the quality of life for SCD patients. Clinical trials aim to validate the promising preclinical findings, offering hope for individuals living with SCD by reducing pain, minimizing complications, and enhancing overall well-being. Furthermore, the research employs phenotypic screening of a small molecule library to identify compounds capable of inhibiting HbS polymerization. By targeting the root cause of SCD, this approach aims to advance drug discovery efforts and develop more effective therapeutic interventions. Thus, this thesis aims to enhance SCD understanding, management, and patient outcomes.

Contents

1. Red Blood Cells as Therapeutic Target to Treat Sickle Cell Disease
2. Investigating metabolic and elemental profiles in sickle cell patients, to unveil pathophysiological mechanisms and therapeutic avenues
3. Phenotypic screening of small molecule library for their potential anti-HbS polymerization efficacy to advance SCD drug
4. Ex-vivo antisickling efficacy and toxicity of Sailin-HbS®, an herbal formulation, in mitigating of RBC biomechanical breakdown in SCD
5. Evaluation of safety,

tolerability and efficacy of Sailin-HbS®: A potent indigenous antisickling agent that reduces pain and improves the quality of life in patients with sickle cell disease 6. Research prospects and appendix.

02. GOSWAMI (Nikita)
Investigating the Role of Downstream Targets of Mycobacterium Tuberculosis Transcriptional Regulator VirS and identification of novel VirS inhibitors.
 Supervisor: Dr. Garima Khare
Th 27350

Abstract

Tuberculosis (TB) is a deadly disease, caused by Mycobacterium tuberculosis (M. tb). Despite the availability of a vaccine and an effective chemotherapy, TB still remains a serious health hazard, afflicting millions of lives every year. M. tb faces various hostile conditions inside the host such as acidic stress, oxidative and nitrosative stress. However, the pathogen has devised strategies to overcome these host mounted stresses. VirS (Rv3082c) is an acid induced mycobacterial transcriptional regulator that has been shown to be involved in the virulence and pathogenesis of M. tb and is considered to be as a crucial drug target. Previous studies in our laboratory showed that VirS regulates a myriad of genes that are involved in various pathways. For this study, we selected two downstream targets of VirS that were upregulated under low pH conditions in a VirS dependent manner namely (i) Rv3177, which is annotated as putative non-heme peroxidase and (ii) KdpC that encodes for the c-subunit of high-affinity K⁺-uptake transporter complex, KdpFABC. In order to understand the importance of the genes in growth and pathogenesis of the pathogen, we employed homologous recombination technique to generate deletion mutants of these genes in M. tuberculosis and further their role was investigated using various in-vitro and in-vivo experiments. Moreover, since VirS is considered to a crucial drug target, we employed a few previously identified VirS inhibitors and identified their analogs. These analogs were evaluated for their inhibitory potential against VirS using various in-vitro and ex-vivo experiments. This study characterized the role of Rv3177 and kdpC in M. tb and led to identification of a few novel inhibitors against M. tb.

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1. Introduction 2. Review of literature 3. Aims and Objectives 4. Results and Discussion 5. To evaluate the role of Rv3177 in the growth and pathogenesis of Mycobacterium tuberculosis 6. To evaluate the role of KdpC in the growth and pathogenesis of Mycobacterium tuberculosis 7. Identification of inhibitory molecules against Mycobacterium tuberculosis, by employing structural analogs of VirS inhibitors. Annexure, Summary and Conclusions, Appendix and Publications.

03. MAHONGNAO (Sophayo)
Formation, Characterisation, and Quality Assessment of Leaf Waste Compost.
 Supervisor: Prof. Sarita Nanda
Th 27351

Abstract

In recent years, environmental sustainability and efficient resource utilisation have become critical concerns. Urbanisation and population growth have led to increased organic wastes, including green waste such as leaf litter, posing significant waste management challenges. Traditional disposal methods, such as landfilling and burning, contribute to pollution and overlook the potential of leaf litter as a nutrient-

rich resource. This research focuses on transforming leaf litter into high-quality bio-compost, through effective microbe (EM) composting, for its potential application as bio-organic fertiliser that can promote environmental sustainability. Our investigation into leaf litter composting over 50 days revealed that compost maturity was achieved between 20 to 40 days, marked by stable pH, electrical conductivity, carbon-to-nitrogen ratio, and total organic carbon content. Moreover, the composting process reduced potentially toxic elements (PTEs) like arsenic, cadmium, chromium, and nickel. Leaf compost demonstrated high fertilising potential with minimal PTE contamination, rich in macronutrients like carbon, nitrogen, and sulfur. The study also highlighted the diverse microbiome richness, with beneficial bacteria like *Lactobacillus*, *Leuconostoc*, *Sphingobacterium*, *Clostridium*, *Acetobacter*, and others, thriving in leaf compost. Fungal microbiome with potential benefits was improved compared to other organic waste composts, with beneficial genera such as *Pichia* and *Geotrichum*. The abundance of beneficial microbiome was observed to be higher, with minimal level of pathogens in the leaf compost, compared to other composts. Incorporating leaf compost as soil amendment could increase soil nutrient levels and reduced PTE concentration, promoting soil fertility and reducing environmental risks. The soil microbiome was also enhanced with reduction of soil borne pathogens due to leaf compost amendment. In conclusion, leaf litter could be effectively transformed into high-quality organic compost, serving as a valuable bio-organic fertiliser. This approach not only addresses waste management issues but also supports sustainable environment and agricultural practices by enhancing soil health and reducing reliance on synthetic fertilisers.

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1. Introduction, Review of Literature, Aims and Objectives 2. Optimisation and characterisation of compost formation from leaf waste through composting 3. Characterisation and comparison of leaf waste compost quality with other types of organic waste compost 4. Characterisation of the microbiome composition in the leaf and other organic composts for its potential application as bio-organic fertilizer 5. Analysing the fertilising and bio-remediation potential of leaf and other organic compost in the soils through indexing method 6. Analysing the Bacterial and Fungal Microbiome Richness and Diversity in the Soils Amended with the Leaf Compost and Other Organic Waste Composts 7. Summary, conclusions and future perspective. Appendix and Publications.

04. NUPUR

Employing Various Drug Discovery Approaches for the Control of Tuberculosis.

Supervisor: Dr. Garima Khare

Th 27352

Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (M. tb), continues to afflict human race since ages. According to the WHO Global TB Report 2023, there were 10.6 million new TB cases and 1.3 million deaths globally due to the disease including 0.6 million deaths by HIV-TB coinfection. The standard TB chemotherapeutic regimen consisting of four frontline drugs namely, rifampicin, isoniazid, pyrazinamide and ethambutol is administered for over 6 months. Non-compliance to this prolonged treatment, emergence of drug resistant strains, side effects associated with the therapy and interference of anti-TB drugs with HIV medication in case of HIV-TB coinfecting patients, emphasize the urgency to identify novel and better drug molecules to develop more efficacious and shorter chemotherapeutic regimen.

Towards the identification of new anti-TB molecules, several approaches have been utilized over the years, which include high-throughput phenotypic screening, target-based virtual screening, repurposing of drugs and modification of existing drug scaffolds. The studies detailed in this thesis are focused on the identification of novel inhibitory molecules against *M. tb* by employing various drug discovery approaches. We demonstrated the importance of a *M. tb* protein, dihydrodipicolinate reductase (DapB, Rv2773c) which is involved in lysine and cell wall biosynthesis of mycobacteria. DapB was validated as a drug target and structure-based virtual screening against DapB was performed to identify novel inhibitors against *M. tb*. Whole cell-based high-throughput phenotypic screening was employed for the identification of inhibitory molecules by screening a library of molecules derived from natural products. We also employed drug repurposing approach by using a library of FDA approved drugs. Thus, by employing various drug discovery approaches, we have identified several hit molecules that lay the foundation for further lead optimization and development of new potent drugs against TB. Additionally, we found an anti-cancer drug with potent anti-TB activity as a repurposed drug for TB.

Contents

1. Acknowledgements 2. Abbreviations 3. Review of literature 4. Aims and Objectives 5. Results and Discussion 6. Evaluation of the role of DapB, an enzyme of diaminopimelate pathway, in the in vitro and intracellular growth of *Mycobacterium tuberculosis* 7. In silico virtual screening for the identification of novel inhibitors against dihydrodipicolinate reductase (DapB) of *Mycobacterium tuberculosis* 8. Identification of novel inhibitors against *Mycobacterium tuberculosis* by utilizing whole cell-based high-throughput phenotypic screening of small molecule libraries. Summary and Conclusions, Appendix and Publications.

05. SHARMA (Pooja)
Quality Assessment of Organic Leafy Vegetables Cultivars Grown with Different Soil Amendments.
 Supervisor: Prof. Sarita Nanda
Th 27866

Abstract

Global demand for organic food is rising, with 191 countries practising organic farming across over 76.4 million hectares of farmland worldwide, and India contributing 43.24% of the global organic producers. Despite this growth, concerns about food safety issues such as pathogens and heavy metals especially those grown on contaminated Yamuna floodplains are still not investigated thoroughly. This study examined the impact of different compost amendments on the quality, safety, and microbial diversity of leafy vegetables grown in contaminated Yamuna floodplain soil, assessing compost as a sustainable alternative to chemical fertilizers. The floodplain cultivars were compared to cultivars grown on garden soil as control. Five compost types viz. leaf waste, municipal waste, cow dung manure, kitchen waste, and vermicompost were applied to *Spinacia oleracea*, *Trigonella foenum-graecum*, *Amaranthus viridis*, and *Amaranthus cruentus*. Analytical techniques, including spectroscopic methods and ICP-MS, were used to assess macrominerals, antioxidants, antinutrients, potentially toxic elements (PTE), and trace elements. Health risk assessments evaluated dietary intake values, hazard indices, and cancer risks. Additionally, 16S rRNA and ITS metagenomic profiling analyzed microbial and fungal diversity. The findings revealed that compost amended cultivars exhibited improved quality and safety, with reduced PTE levels and increased macrominerals and antioxidant concentrations. Health risk assessments indicated lower dietary

intake values, hazard indices, and cancer risks in compost amended crops. Enhanced microbial diversity, with a greater presence of beneficial bacterial and fungal microorganisms and fewer pathogens, was also observed. These results suggest that compost amendments can sustainably improve the quality of the cultivars, providing a viable alternative to chemical fertilizers in vulnerable regions like the contaminated Yamuna floodplain. The study highlights the importance of sustainable, environmentally friendly agricultural practices for food quality and public health.

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1. Introduction, Review of Literature, Aims and Objectives 2. Assessing the mineral, trace and potentially toxic element profiles of compost-amended crops cultivated in the Yamuna river floodplain soil 3. The relationship between PTE and the presence of antioxidants and also antinutrients in crops cultivated in compost-enriched soil from the Yamuna floodplain 4. Bacterial and Fungal microbiome of the compostamended red amaranth cultivated in the Yamuna river floodplain soil 5. Summary, Conclusion and Future perspective. Appendix and publications.