# Contents

<table>
<thead>
<tr>
<th>Acknowledgement</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents</td>
<td>IV</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>IX</td>
</tr>
<tr>
<td>Preface</td>
<td>X</td>
</tr>
</tbody>
</table>

## 1. Introduction

1.1. Discovery of viruses  
1.2. Bacteriophage  
1.3. Bacteriophage λ  
1.3.1. Organization of the λ genome  
1.3.2. Life cycle of bacteriophage λ  
1.3.2.1. Infection to host  
1.3.2.2. Expression of early genes  
1.3.2.3. Antitermination and expression of delayed early genes  
1.3.2.4. Switching of developmental pathway and lysogeny  
1.3.2.5. Lytic development  
1.3.2.5.1. Expression of the late genes  
1.3.2.5.2. Replication of the λ DNA  
1.3.2.5.3. Assembly of the phage particle  
1.3.2.5.4. Lysis of the host  
1.3.2.6. Lysogenic development of bacteriophage λ: Establishment and maintenance of lysogeny  
1.3.2.7. Molecular nature of the operators  
1.3.2.8. Interaction of the repressor with single operator sites $O_R l$  
1.3.2.9. Repressor induced DNA bending in the $O_R l$ region  
1.3.2.10. Repressor induced DNA looping  
1.3.2.11. In vivo repressor concentration  
1.3.2.12. Structure – function relationship of the λ repressor  
1.3.2.13. Equilibrium and kinetics of λ repressor – operator interaction  

Page No.
1.4. Lysogenic development of bacteriophage P1 25
1.5. Lysogenic development of B. subtilis phage φ105 26
1.6. Mycobacteriophages 31
1.6.1. Classification of mycobacteriophages 31
1.6.2. Morphology 31
1.6.3. Host specificity 31
1.6.4. Analysis of mycobacteriophage genomes 32
1.6.5. Genes involved in mycobacteriophage-mediated lysis of host 34
1.6.6. Factors involved in the integration of temperate mycobacteriophage genomes 34
1.6.7. Other gene functions 34
1.6.8. Regulation of gene expression of mycobacteriophage 35
1.6.9. Molecular characterization of the repressor genes of mycobacteriophages 35
1.6.9.1. Cloning and characterization of repressor gene of mycobacteriophage L5 35
1.6.9.2. Cloning and characterization of repressor gene of mycobacteriophage Bxb 1 38
1.6.9.3. Cloning and characterization of repressor gene of mycobacteriophage L1 38
1.6.9.3.1. Identification, cloning and sequencing of L1 repressor gene 38
1.6.9.3.2. Cloning of the promoters of mycobacteriophage L1 40
1.6.9.3.3. Operator DNA binding activity of wild-type and ts repressor proteins 40
1.6.9.3.4. Effect of physical and ionic factors on the activity of the repressor 42
1.6.9.3.5. Domain structure of L1 repressor 44
1.6.9.3.6. Secondary structure of His-Cl and the C-terminal domain (CTD) 46
1.6.9.3.7. Oligomeric status of L1 repressor 46
1.6.9.3.8. Interaction of His-Cl with $O_{64}$ and $O_{L}$ operators 46
2. Objectives 51
3. Materials and methods 53
3.1. Materials 53
3.1.1. Non-radioactive chemicals 53
3.1.2. Radioactive chemicals 53
3.1.3. Filter papers 53
3.1.4. Enzymes, Markers and Kits 53
3.1.5. Media, their compositions and solutions 53
3.1.6. Bacterial & phage strains, plasmids and oligonucleotides 57
3.2. Methods 57
  3.2.1. Growth of bacteria 57
  3.2.2. Preparation of L1 phage 57
    3.2.2.1. Plate lysis method 57
    3.2.2.2. Broth lysis method 58
    3.2.2.3. Assay of phage titre 58
  3.2.2.4. Determination of plating efficiency of phages on bacteria 58
  3.2.3. Preparation of L1 DNA 58
    3.2.3.1. Concentration of L1 phage 58
    3.2.3.2. Isolation of L1 DNA 59
  3.2.4. Isolation of plasmid DNA 59
    3.2.4.1. Alkaline lysis method 59
    3.2.4.2. Isolation of plasmid from *E. coli* by boiling prep method 60
    3.2.4.3. Isolation of plasmid from *M. smegmatis* 60
  3.2.5. Digestion of DNA by restriction endonucleases 61
  3.2.6. Agarose gel electrophoresis 61
  3.2.7. Purification of DNA from agarose gel 61
  3.2.8. Modification of DNA 62
    3.2.8.1. Ligation of protein 62
    3.2.8.2. Labeling of 5' end of DNA 62
    3.2.8.3. Klenow fill up of recessed 3' termini of DNA 62
  3.2.9. DNA amplification by polymerase chain reaction (PCR) 62
  3.2.10. Transformation of DNA 63
    3.2.10.1. CaCl₂ method 63
    3.2.10.2. Electrotransformation method 63
  3.2.11. Construction of plasmids 63
  3.2.13. Overexpression and purification of the repressors 70
3.2.13.1. Overexpression and purification of MBP-CI 70
3.2.13.2. Overexpression and purification of His-tagged repressors 70
3.2.14. Estimation of proteins 71
3.2.15. Purification of C-terminal domain (CTD) of L1 repressor 71
3.2.16. Preparation of DNA fragments for the bending and genetic investigations 72
3.2.17. Polyacrylamide gel electrophoresis (PAGE) 72
3.2.17.1. Tris-glycine SDS-PAGE 72
3.2.17.2. Native PAGE 75
3.2.17.3. Urea PAGE 75
3.2.17.4. Staining of polyacrylamide gels with commassie brilliant blue 75
3.2.17.5. Staining of polyacrylamide gels with silver nitrate 76
3.2.17.6. Drying polyacrylamide gel 76
3.2.17.7. Autoradiographing and developing 76
3.2.18. Gel-shift assay 76
3.2.19. Mixed oligomerization assay 78
3.2.20. Circular dicroism (CD) spectrum 78
3.2.21. Thermal aggregation 78
3.2.22. Tryptophan fluorescence spectra 78
3.2.23. Chemical crosslinking 78
3.2.24. Labeling of top and bottom strands of O64 DNA 79
3.2.25. DMS protection assay 79
3.2.26. Hydroxyl radical footprinting 79
3.2.27. In vivo repressor concentration determination by western blot 80
3.2.28. Limited proteolysis at 32°C and 42°C 80
3.2.29. DTNB assay 81
3.2.30. Bioinformatic analysis 81
3.2.31. Statistical analysis 82
4. Results and discussion 83
4.1. Results 83
4.1.1. Effect of different ions on the DNA binding capacity of L1 repressor 83
4.1.1.1. Effect of monovalent cations on the structure and function

VII
of LI repressor 83
4.1.1.2. Effect of anions on the structure and function of L1 repressor 86
4.1.1.3. Effect of Mg$^{2+}$ on structure, function and stability of L1 repressor 86
4.1.1.4. Effect of Na$^+$ and Mg$^{2+}$ on the conformation and stability of CTD 90
4.1.2. Studies on the LI repressor - operator interaction 93
4.1.2.1. Bases, strands and grooves of $O_{64}$ DNA helix interacting with Cl 93
4.1.2.2. Operator mutations affecting repressor binding 95
4.1.2.3. Stoichiometry of L1 repressor – operator interaction 95
4.1.2.4. Repressor induced bending of $O_{64}$ DNA 100
4.1.2.5. Determination of in vivo concentration of L1 repressor 100
4.1.3. Mutations affecting the DNA binding activity, structure and stability of L1 repressor 104
4.1.3.1. Construction and purification of different L1 repressor mutants 104
4.1.3.2. In vitro DNA binding activity of the repressors 104
4.1.3.3. In vivo DNA binding activity of the repressors 107
4.1.3.4. Domain structure of the repressors 107
4.1.3.5. Aggregation of the mutant repressors 110
4.1.3.6. Dimerization efficiency of the mutant repressor proteins 110
4.1.3.7. Structures of the repressor proteins 113
4.1.3.8. Cysteine accessibility in His-CIW69C 113
4.2. Discussion 115
5. Summary 124
6. References 126
7. Publications 141