
CONTENTS

A. SUMMARY	1
B. INTRODUCTION	5
C. MATERIALS.....	16
1. BACTERIAL STRAINS	16
2. VECTORS AND PLASMIDS.....	16
3. OLIGONUCLEOTIDES.....	19
4. MEDIA.....	20
5. AGAR PLATES	21
6. CHEMICALS AND ENZYMES	22
7. INSTRUMENTS	23
D. METHODS	24
1. SMALL AND MIDI SCALE ISOLATION OF PLASMID DNA FROM <i>E. COLI</i>	24
2. SMALL AND MIDI SCALE PREPARATION OF CHROMOSOMAL DNA FROM <i>HELICOBACTER PYLORI</i>	24
3. CLEAVAGE OF DNA WITH RESTRICTION ENDONUCLEASES	25
4. FILLING IN OF DNA ENDS WITH KLENOW ENZYME.....	26
5. 5'-DEPHOSPHORYLATION OF DNA ENDS WITH ALKALINE PHOSPHATASE.....	26
6. POLYMERASE CHAIN REACTION.....	26
7. DEPROTECTION OF OLIGONUCLEOTIDES	27
8. ANALYSIS OF DNA FRAGMENTS BY AGAROSE GEL ELECTROPHORESIS	27
9. SOUTHERN BLOT	27
10. ISOLATION OF DNA FRAGMENTS FROM AGAROSE GELS	28
11. CLONING OF DNA FRAGMENTS.....	28
12. PREPARATION OF COMPETENT CELLS OF <i>E. COLI</i> STRAINS DH5 α AND BL21.....	28
13. TRANSFORMATION OF <i>E. COLI</i> DH5 α AND BL21	29
14. TRANSFORMATION OF <i>HELICOBACTER PYLORI</i> STRAINS	29
15. LONG TERM STORAGE OF BACTERIAL STRAINS	29
16. β -GALACTOSIDASE ASSAY	30
17. PURIFICATION OF RECOMBINANT PROTEINS	30
18. PURIFICATION OF THE WILD TYPE AND THE C-TERMINAL TRUNCATED FORM OF THE <i>E. COLI</i> RNA POLYMERASE α SUBUNIT.....	31
19. ANALYSIS OF PROTEINS BY SDS POLYACRYLAMIDE GEL ELECTROPHORESIS	31
20. COOMASSIE BLUE STAINING OF SDS-POLYACRYLAMIDE GELS.....	32
21. IMMUNOBLOT (WESTERN BLOT) ANALYSIS	33
22. ANALYSIS OF RADIOACTIVELY LABELED DNA FRAGMENTS BY UREA POLYACRYLAMIDE GEL ELECTROPHORESIS ..	33
23. RNA PREPARATION.....	34
24. 5'-LABELING OF OLIGONUCLEOTIDES	35
25. PRIMER EXTENSION ANALYSIS.....	35
26. <i>IN VITRO</i> TRANSCRIPTION	36
27. LABELING OF DNA FRAGMENTS AT SPECIFIC RESTRICTION SITES	36
28. S1 NUCLEASE MAPPING	36
29. SEQUENCING OF CLONED DNA FRAGMENTS	37
30. END LABELING OF DNA FRAGMENTS WITH KLENOW ENZYME	37
31. ELECTROPHORETIC MOBILITY SHIFT ASSAY	37
32. DNASE I FOOTPRINTING.....	38
33. G+A SPECIFIC CLEAVAGE OF END LABELED DNA FRAGMENTS	38

E. RESULTS.....	40
1. TRANSCRIPTIONAL ANALYSIS OF THE DIVERGENT CAGAB GENES ENCODED BY THE <i>H. PYLORI</i> PATHOGENICITY ISLAND.....	40
1.1 Determination of transcription start sites.....	40
1.2 Specificity of promoter recognition	42
1.3 Deletion analysis of the P_1 and P_2 promoter regions	43
1.4 Transcriptional activity of the P_2 and P_3 promoters in the different mutants.....	48
1.5 Binding of the α subunit of RNA polymerase to sequences upstream of the P_1 promoter	50
2. TRANSCRIPTIONAL REGULATION OF FLAGELLAR GENES.....	52
2.1 Identification of flagellar genes transcribed by σ^{54} promoters.....	52
2.2 Identification of the transcriptional activator of the σ^{54} -dependent genes and operons.....	54
2.3 Transcriptional analysis of the <i>flgR</i> gene	56
2.4 <i>FlgR</i> as the master regulator of basal body and hook genes	57
2.5 Binding of <i>FlgR</i> to the <i>flaB</i> promoter	58
2.6 Effect of <i>FlgR</i> on <i>flaA</i> transcription	60
2.7 Effect of DNA topology on <i>flaB</i> transcription.....	61
3. TRANSCRIPTIONAL REGULATION OF CHAPERONE GENES	62
3.1 Determination of transcriptional start sites.....	62
3.2 Identification of the transcriptional regulator of the chaperone genes	64
3.3 Effect of <i>HspR</i> on cellular levels of <i>GroEL</i> and urease	66
3.4 Derepression of P_{gro} and P_{cbp} promoters by osmotic shock	66
3.5 Binding of <i>HspR</i> to the P_{gro} promoter	68
3.6 Differential binding of <i>HspR</i> to the P_{gro} , P_{cbp} and P_{hrc} promoters.....	69
F. DISCUSSION.....	72
1. PATHOGENICITY ISLAND	72
2. FLAGELLA.....	74
3. CHAPERONES	78
G. CONCLUSIONS	81
H. REFERENCES.....	84
I. ABBREVIATIONS.....	96
J. CURRICULUM VITAE	98