

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

GENETIC RESTRICTION IN
ESCHERICHIA COLI STRAIN W

A thesis
presented in partial fulfilment
of the requirements for the degree of
Master of Science
in Microbiology
at
Massey University

A.F. JAMIESON

1971

ABSTRACT

The ability of various phages to propagate on Escherichia coli strain W was investigated. Phages P1, T2, T3, T4, T5, T6 and T7 could not be shown to form plaques on this strain. Phages T2, T3, T5 and T6 prevented the development of a bacterial lawn when added to a plate at an input ratio of about three phage per bacterium; it appears these phages exerted a killing effect on strain W. Phage P1 and phage T4 did not exhibit this killing effect. Phage T1 formed atypical plaques on strain W with an efficiency of plating of 10^{-4} ; it appears these plaques are due to mutants occurring in the T1 population able to propagate on strain W.

All of the above phages adsorbed efficiently to strain W with the exception of T4, explaining its inability to either propagate on or kill strain W. The infection of strain W by P1 was similar in most respects to that of λ but in order to establish the occurrence of conventional restriction, DNA degradation would need to be demonstrated.

Phages were isolated which propagate on strain W; they are similar in morphology to phages T5 and λ and do not readily adsorb to E coli strains B, C or K.

The supernatant from broth cultures of strain W was shown to contain two closely related phages, one plating on E coli C, and the other on E coli K. Each possesses a characteristic pattern of plating efficiencies on strains C and K when propagated alternately in these two hosts but the two phages were shown to be co-immune and identical with respect to heat sensitivity, morphology and serology.

Both tended to lose the ability to exclude phage P1 on lysogenising strain C once having mutated to plate on strain K. This may be due to the integration of the mutated phage at alternate "non-restricting" sites on the E coli C chromosome.

A series of conjugal crosses was employed to determine the sites of integration of the phages on the chromosomes of E coli strains W and C. The phage present in the W supernatant which plated on K was found to integrate close to the proline loci on the chromosome of E coli W but the phage plating on C appeared to have more than one locus, one of which may map close to the 85 minute mark on the linkage map of E coli W (36, Figure 22). No information has so far been obtained concerning the sites of integration of the w phages in restrictive and non-restrictive lysogens of E coli C. The failure to obtain a 'cured' strain of E coli W by elimination of the prophage integrated at the two mapped sites leaves open the possibility of the existence of more than one integration site of phage w.C on the E coli W chromosome.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Professor D.F. Bacon for his invaluable guidance throughout the course of this work and also for the supply of many of the bacterial and bacteriophage strains.

Thanks are also due to the staff of the Palmerston North D.S.I.R. electron microscope unit for their assistance in the preparation of the electron micrographs, to Miss J. Quigan for her expert typing and to all staff in the Microbiology and Genetics Department who have helped in work leading to the presentation of this thesis.

A.F. JAMIESON

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	9
I Superinfection immunity	9
II Superinfection exclusion	9
III Inhibition by F ⁺	10
IV Restriction	11
V Abortive infection by T5 and the T-even phages ..	26
 AIMS OF THE INVESTIGATION	 31
 BACTERIA	 32
 BACTERIOPHAGES	 36
 MEDIA	 37
 METHODS	 40
 RESULTS AND DISCUSSION	 47
I Investigation of the abortive infection of <u>Escherichia coli</u> W by various phages	47
II Isolation of phage able to propagate on <u>Escherichia coli</u> W	63
III Plating efficiencies of phage w on <u>E coli</u> strains C and K	69
IV Comparison of the properties of phages w.KC and w.CKC	75
V Mapping of the sites of integration of the w prophages in the chromosomes of <u>E coli</u> strains C and W ..	87
 CONCLUSIONS	 100
 BIBLIOGRAPHY	 106

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Efficiency of plating of phage w on <u>Escherichia coli</u> strains K and C	22
2. Efficiency of plating of phage w on <u>Escherichia coli</u> strains K and C	22
3. Bacterial strains	32
4. Bacteriophages	36
5. Plating effects at different input levels of phage w on <u>E coli</u> strain W	48
6. Determination of the frequency of productive infection of <u>E coli</u> W cells by phage T7.B	51
7. Efficiencies of plating of phages on <u>Escherichia coli</u> strains W, B and C	55
8. Adsorption coefficients of the T phages and P1 on adsorption to <u>E coli</u> strains W, B and K12	57
9. Screening of <u>E coli</u> isolates and sewage samples for the presence of phage able to propagate on strain W	63
10. Dimensions of the V-phages	66
11. The relative efficiencies of plating of phage from an <u>E coli</u> W supernatant on <u>E coli</u> strains C and K	71
12. Adsorption coefficients of w phage to <u>E coli</u> strains C and K	72
13. Heat sensitivities of phages w.CKC and w.KC in 0.1M NaCl + 0.5M CaCl ₂	75
14. Heat sensitivities of phages w.CKC and w.KC in 0.1M NaCl	75
15. Adsorption of phages w and P1 to lysogens of <u>E coli</u> C	81
16. Ability of <u>E coli</u> K and C lysogenised with phage w to support the propagation of various phages	82
17. Abortive infection of phage w lysogens of C by phage P1	83
18. Presence of phage able to plate on strains K and C in the supernatants of broth cultures of substrains of <u>E coli</u> W	88

<u>Table</u>	<u>Page</u>
19. Frequency of selected and unselected markers in the cross Hfr H x D2-18-1-0 ⁺	90
20. Frequency of selected and unselected markers in the cross Hfr H x D2-8	91
21. Frequency of selected and unselected markers in the cross Hfr F2-3-27 x C1-a/50	93
22. Frequency of selected and unselected markers in crosses of Hfr H with lysogens of <u>E coli</u> strains C and K	96
23. Frequency of recovery of selected and unselected markers in the cross Hfr 808 x D2-18-1-0 ⁺ (pro ⁺ w.K ⁻) ..	98

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Restriction and modification of phage λ in <u>Escherichia coli</u> strains K and C	12
2. The specificity site nucleotide sequence of <u>Haemophilus influenzae</u>	16
3. Derivation of substrains of <u>Escherichia coli</u> strain W (D.F. Bacon)	33
4. Linkage map of <u>Escherichia coli</u> K12	34
5. Adsorption kinetics of phage T1 to <u>Escherichia coli</u> strains B and W	58
6. Adsorption kinetics of phage T2 to <u>Escherichia coli</u> strains B and W	58
7. Adsorption kinetics of phage T3 to <u>Escherichia coli</u> strains B and W	59
8. Adsorption kinetics of phage T4 to <u>Escherichia coli</u> strains B and W	59
9. Adsorption kinetics of phage T4 to <u>Escherichia coli</u> strains B and C	60
10. Adsorption kinetics of phage T5 to <u>Escherichia coli</u> strains B and W	60
11. Adsorption kinetics of phage T6 to <u>Escherichia coli</u> strains B and W	61
12. Adsorption kinetics of phage T7 to <u>Escherichia coli</u> strains B and W	61
13. Adsorption kinetics of phage P1 to <u>Escherichia coli</u> strains K and W	62
14. Plating efficiencies of phage from the supernatant of <u>E coli</u> W on strains K and C	70
15. Adsorption kinetics of phage w.K to <u>E coli</u> strains C and K	73
16. Adsorption kinetics of phage w.KC to <u>E coli</u> strains C and K	73
17. Adsorption kinetics of phage w.C to <u>E coli</u> W strains C and K	74

<u>Figure</u>	<u>Page</u>
18. Kinetics of Heat Inactivation of phage w.CKC at 70°C in 0.1M NaCl + 0.5M CaCl ₂	76
19. Kinetics of Heat Inactivation of phage w.KC at 70°C in 0.1M NaCl + 0.5M CaCl ₂	76
20. Inactivation of phage w.CKC and phage w.KC by w.CKC antiserum	79
21. Inactivation of phage w.CKC and phage w.KC by w.KC antiserum	80
22. The tentative locations on the chromosome of <u>E coli</u> W of: i) the W genome <u>hs</u> site; ii) two possible integration sites of phage w	95

LIST OF PLATES

<u>Plate</u>	<u>Page</u>
1. Plaques formed by phage T1.B on <u>E coli</u> B (x3) ..	53
2. Plaques formed by phage T1.B on <u>E coli</u> W (x3) ..	53
3. Plaques formed by phage T2.B on <u>E coli</u> B (x3) ..	56
4. Plaques formed by phage T2.B on <u>E coli</u> C (x3) ..	56
5. The screening method used to detect phage and colicins active against <u>E coli</u> W	64
6. Plaques formed by phage V11 on <u>E coli</u> W (x3) ..	64
7. Phage V7 (x116,000)	67
8. Phage V10 (x116,000)	67
9. Phage V11 attached to bacterial debris (x116,000)	67
10. Phage V11 attached to cell debris (x116,000) ..	67
11. Phage V11 adsorbed to <u>E coli</u> W; approximate phage input ratio = 50:1 (x85,000)	68
12. Phage V11 and <u>E coli</u> K; approximate phage input ratio = 50:1 (x48,000)	68
13. A rosette of phage w (x116,000)	78
14. Phage w.KC (x116,000)	78
15. Phage w.CKC (x116,000)	78
16. Plaques of phage w.CKC on <u>E coli</u> C (x3) ..	85
17. Screening of lysogens for ability to exclude phage P1	85
18. A lysogen of <u>E coli</u> K K(w.CK) spotted on a plate previously spread with phage P1 particles ..	86
19. The method employed to screen for phage w lysogens of <u>E coli</u> strains K and C	86
