STUDIES OF BACTERIOPHAGES INDUCED FROM *STREPTOCOCCUS CREMORIS* STRAIN R_1: IS R_1 A DOUBLE LYSOGEN ?

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Genetics at Massey University, New Zealand.

Sin Hen Phua

1979

ABSTRACT

Early studies on *Streptococcus cremoris* strain R_1 suggested that it was polylysogenic. Later, it was reported that its induced lysates contained bacteriophages (phages) of two types which were believed to differ in their morphology, buoyant densities, immune specificities and in their responses to heterologous antiphage sera. Further work on the strain did not reproduce the above observations, but did often give results which were consistent with it being a double lysogen. This project was an in-depth investigation of phages induced from R_1, in an attempt to establish the single or double lysogenic nature of the strain.

Mid-log phase R_1 cells were harvested, washed with homologous antiphage serum and induced to lyse with ultraviolet light (UVL). The resulting phage lysates were analysed on caesium chloride (CsCl) density gradients. Though the OD_{254} (optical density at 254 nm) scans of the gradients detected the presence of only one phage band, p.f.u. (plaque forming unit) profiles of the gradient fractions on indicator strains R_1C and 368 revealed, in addition to the main phage peak, several minor p.f.u. peaks (termed satellite and shoulder peaks) as possible manifestations of different phage types in the R_1 lysates. Further CsCl density gradient analyses of phage stocks and pooled phage fractions of these minor p.f.u. peaks showed that the latter phages were identical with those of the main phage peaks of mean buoyant density of 1.485 g/ml.

Further characterization of the phages recovered from the CsCl gradients by neutralization tests with homologous antiphage serum confirmed the existence of only one serological phage type in the R_1 lysates. Final verification of the unity in phage type in R_1 lysates came from SDS-gel electrophoreses of the phages recovered from the different p.f.u. peaks and from lysates, which showed the largely identical gel patterns of their protein components. Host-specificity tests of the phages provided the last piece of evidence for the conclusion that R_1 is a single lysogen, harbouring only one prophage in its genome. Review of past electron-microscopic studies

of R_1 lysates substantially support this conclusion. In fact, reconstruction of R_1 by lysogenization of a cured strain (R_1C) yielded a strain (R_1r) which closely resembled the original in lysogenic properties.

From the data collected in the course of this work, it was inferred that 368 lysates possibly contained defective phages. An attempt was made to cure 368 of its supposedly defective prophage in the hope of providing a 'cleaner' strain for studying the host-induced variation observed in the R_1C-368 system. Though possible cured derivatives were obtained, they did not prove to be an improvement over the parental strain 368 with respect to their efficiency of plating for R_1 phages.

Finally, phage mutant isolation and recombination experiments were attempted in the hope of gaining an insight into the lysogenic system operating in the R_1 cells. Using UVL and nitrous acid (HNO_2) mutagenesis on the temperate $\phi r_1/R_1C$ induced from R_1, about 75 independently arising clear plaque-forming mutants were isolated for mapping experiments. Pairwise crosses between the UVL and HNO_2^- induced mutants were performed by coinfecting R_1C cells. Though far from conclusive, the preliminary results obtained indicated a general low occurrence of turbid-plaqued (wild type) phage recombinants, and hence a low frequency of recombination.

ACKNOWLEDGEMENTS

I am indebted to the Department of Microbiology and Genetics, Massey University, for providing the space and the facilities.

Especially, I would like to convey my thanks to Prof. Donald F. Bacon for his acceptance, and the patience and understanding he has shown me; to all the departmental staff for their endurance and the friendships they so willingly offered which made my stay an enjoyable one; to Mr Robert W. Cleaver for expert technical assistance; to Dr John W. McLean for tit-bits on whereabouts to purchase the necessary stationeries; and to the members of the Journal Club and the Molecular Biology - Biochemistry Research Group for many worthwhile lunch-time sessions.

My thanks also to Mrs M. McAusland, Mrs A. Prichard and Mrs C. Taylor for typing the first drafts, and special thanks to Mrs Fay S. Wicherts for her excellent final typing and professionalism.

It is difficult for me to put in words the gratitude and appreciation I have toward my supervisor, Dr Eric A. Terzaghi. His unfailing help, enthusiasm, patience and concern, and his invaluable guidance and advice through all my years at Massey will always be remembered. As a lecturer, supervisor and personal friend, he is unique.

TABLE OF CONTENTS

Page

ABSTRACT ii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF TABLES vii

LIST OF FIGURES viii

LIST OF PLATES x

SECTION ONE: INTRODUCTION 1

SECTION TWO: MATERIALS

1. Bacteria 15
2. Bacteriophages 15
3. Media 16
4. Other solutions 19

SECTION THREE: EXPERIMENTAL PROCEDURES

1. Maintenance of lactic streptococcal bacteria 25
2. Assay, isolation and propagation of lactic streptococcal phages 26
3. Preparation of indicator *Escherichia coli* strains for assay of coliphages 28
4. Measurement of growth kinetics in broth cultures of lactic streptococci 29
5. Ultraviolet light irradiation of bacteria 30
6. Ultraviolet light irradiation of phages 31
7. Nitrous acid mutagenesis of phages 32
8. Ultraviolet light induction of bacteria 33
9. Caesium chloride density gradient equilibrium run 35
10. Neutralization of phages by antiphage serum 36
11. SDS-gel electrophoresis of phage proteins 37
12. Spot tests for testing susceptibility of bacterial strains to phages 45
13. Experiments on curing of *Streptococcus cremoris* strain 368 46
14. General procedure used in phage recombination experiments 50

SECTION FOUR: RESULTS AND DISCUSSIONS

		Page
1.	Some characteristics of the lactic streptococcal bacteria	53
2.	Ultraviolet light irradiation of lactic streptococcal bacteria	56
3.	Ultraviolet light induction of *Streptococcus cremoris* strains R_1, R_1r, 368 and 368(r_1)	56
4.	Electron-microscopic studies of phage lysates and stocks	62
5.	Caesium chloride density gradient analysis of R_1 phages	64
6.	Serological tests of R_1 phages against antiphage serum A/S r_1-UV1/R_1C	79
7.	SDS-gel electrophoresis of R_1 phage proteins	97
8.	Host-specificity tests of R_1 phages on different strains of lactic streptococci	102
9.	An attempt at curing of *Streptococcus cremoris* strain 368	107
10.	Ultraviolet light and nitrous acid mutagenesis of phages, and phage recombination experiments	109

SECTION FIVE: CONCLUSION 115

BIBLIOGRAPHY 120

LIST OF TABLES

		Page
I	Some physiological tests used to differentiate the streptococci	3
II	Group classification of the streptococci	5
III	Differential physiological characteristics of Group N streptococci	9
IV	Some distinguishing characteristics of *Streptococcus cremoris* strains R_1, R_1C, R_1r, 368 and 368(r_1)	54
V	Data on ultraviolet light induction of *Streptococcus cremoris* strains R_1, R_1r, 368 and 368(r_1)	61
VI	Data on caesium chloride density gradient analysis of R_1 phages	66
VII	Occurrence of shoulder peak in caesium chloride runs of R_1 phages	86
VIII	Spectrum of lytic response of lactic streptococcal strains to different induced lysates	103
IX	Susceptibility of lactic streptococcal strains to phages isoalted from caesium chloride run of R_1 lysate B	104
X	Efficiency of plating of phages on *Streptococcus cremoris* strains R_1C and 368	106

LIST OF FIGURES

Page

1. Growth curves of 1% broth cultures of *Streptococcus cremoris* strains R_1, 368 and 368(r_1) at 30°C 55

2. Ultraviolet light survival curves of *Streptococcus cremoris* strains 368 and 368(r_1) 57

3. First ultraviolet light induction curve of *Streptococcus cremoris* strain R_1 58

4. Ultraviolet light induction curves of *Streptococcus cremoris* strains R_1, R_1r, 368 and 368(r_1) 59

5. Caesium chloride run of R_1 lysate A 67

6. Caesium chloride run of Peak AI/R_1C stock from R_1 lysate A 69

7. Caesium chloride run of Peak AI/368 stock from R_1 lysate A 70

8. Caesium chloride run of Peak AII/R_1C stock from R_1 lysate A..... 71

9. Caesium chloride run of Peak AIII/R_1C stock from R_1 lysate A 72

10. Caesium chloride run of Peak AIII/368 stock from R_1 lysate A 73

11. Caesium chloride run of R_1r lysate 75

12. Caesium chloride run of 368(r_1) lysate 76

13. Caesium chloride run of R_1 lysate B 78

14. Caesium chloride run of Peak BI fractions from R_1 lysate B 80

15. Caesium chloride run of Peak BII fractions from R_1 lysate B 81

16. Caesium chloride run of Peak BI/R_1C stock from R_1 lysate B 82

17. Caesium chloride run of Peak BI/368 stock from R_1 lysate B 83

18. Caesium chloride run of Peak BII/R_1C stock from R_1 lysate B 84

19. Caesium chloride run of Peak BII/368 stock from R_1 lysate B 85

20. Neutralization of ør_1/R_1C and of ør_1/368 by A/S r_1-UV1/R_1C at 30°C 88

21. Neutralization of ør_1-UV1/R_1C by A/S r_1/368 at 30°C 89

22. Caesium chloride run of ør_1-UV1/R_1C stock 90

Page

23. Neutralization of phages from caesium chloride run of ϕr_1-UV1/R_1C stock by A/S r_1-UV1/R_1C at 30°C 91

24. Neutralization kinetics of phages by homologous antiphage sera at 30°C and at 37°C 92

25. Neutralization of phages from caesium chloride run of R_1 lysate A by A/S r_1-UV1/R_1C at 30°C 93

26. Neutralization of phages from caesium chloride run of R_1r lysate by A/S r_1-UV1/R_1C at 30°C 94

27. Neutralization of phages from caesium chloride run of 368(r_1) lysate by A/S r_1-UV1/R_1C at 30°C 95

28. Neutralization of phages from caesium chloride run of R_1 lysate B by A/S r_1-UV1/R_1C at 37°C 96

29. Ultraviolet light inducibility tests of 'cured' derivatives of *Streptococcus cremoris* strain 368 108

30. Ultraviolet light irradiation of temperate $\phi r_1/R_1$C 111

31. Nitrous acid treatment of temperate $\phi r_1/R_1$C 112

32. Ultraviolet light and nitrous acid survival curves of the clear plaque-forming mutant, ϕr_1-UV1/R_1C 113

33. Flow-chart summary of bacteria and phages, and the scheme of analyses used 119

LIST OF PLATES

		Page
A	SDS-gel electrophoresis of phage stocks and lysates	98
B	SDS-gel electrophoresis of phages isolated from caesium chloride run of R_1 lysate B	99
C	SDS-gel electrophoresis of ultraviolet light induced lysates	100