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STUDIES OF BACTERIOPHAGES INDUCED FROM
STREPTOCOCCUS CREMORIS STRAIN R₁:
IS R₁ A DOUBLE LYSOGEN ?

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ABSTRACT

Early studies on *Streptococcus cremoris* strain R₁ 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 R₁, in an attempt to establish the single or double lysogenic nature of the strain.

Mid-log phase R₁ 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 OD₂₅₄ (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 R₁C 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 R₁ 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 R₁ lysates. Final verification of the unity in phage type in R₁ 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 R₁ 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-plaques (wild type) phage recombinants, and hence a low frequency of recombination.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF PLATES	x
<u>SECTION ONE:</u> INTRODUCTION	1
<u>SECTION TWO:</u> MATERIALS	
1. Bacteria	15
2. Bacteriophages	15
3. Media	16
4. Other solutions	19
<u>SECTION THREE:</u> EXPERIMENTAL PROCEDURES	
1. Maintenance of lactic streptococcal bacteria.....	25
2. Assay, isolation and propagation of lactic streptococcal phages	26
3. Preparation of indicator <i>Escherichia coli</i> 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 <i>Streptococcus cremoris</i> strain 368	46
14. General procedure used in phage recombination experiments	50

<u>SECTION FOUR:</u>	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 <i>Streptococcus cremoris</i> 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 ₁ C	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 <i>Streptococcus cremoris</i> strain 368	107
10.	Ultraviolet light and nitrous acid mutagenesis of phages, and phage recombination experiments ...	109
 <u>SECTION FIVE:</u>		
	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 <i>Streptococcus cremoris</i> strains R ₁ , R ₁ C, R ₁ r, 368 and 368(r ₁)	54
V	Data on ultraviolet light induction of <i>Streptococcus cremoris</i> strains R ₁ , R ₁ r, 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 <i>Streptococcus cremoris</i> strains R ₁ C and 368	106

LIST OF FIGURES

	Page
1. Growth curves of 1% broth cultures of <i>Streptococcus cremoris</i> strains R ₁ , 368 and 368(r ₁) at 30°C	55
2. Ultraviolet light survival curves of <i>Streptococcus cremoris</i> strains 368 and 368(r ₁)	57
3. First ultraviolet light induction curve of <i>Streptococcus cremoris</i> strain R ₁	58
4. Ultraviolet light induction curves of <i>Streptococcus cremoris</i> 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 ₁ C 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 ₁ C stock from R ₁ lysate A.....	71
9. Caesium chloride run of Peak AIII/R ₁ C 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 ₁ C 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 ₁ C 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 $\phi r_1/R_1C$ and of $\phi r_1/368$ by A/S r ₁ -UV1/R ₁ C at 30°C	88
21. Neutralization of ϕr_1 -UV1/R ₁ C by A/S r ₁ /368 at 30°C	89
22. Caesium chloride run of ϕr_1 -UV1/R ₁ C stock	90

	Page
23. Neutralization of phages from caesium chloride run of ϕr_1 -UV1/R ₁ C stock by A/S r ₁ -UV1/R ₁ C 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 ₁ C at 30°C	93
26. Neutralization of phages from caesium chloride run of R ₁ r lysate by A/S r ₁ -UV1/R ₁ C at 30°C	94
27. Neutralization of phages from caesium chloride run of 368(r ₁) lysate by A/S r ₁ -UV1/R ₁ C at 30°C	95
28. Neutralization of phages from caesium chloride run of R ₁ lysate B by A/S r ₁ -UV1/R ₁ C at 37°C	96
29. Ultraviolet light inducibility tests of 'cured' derivatives of <i>Streptococcus cremoris</i> strain 368	108
30. Ultraviolet light irradiation of temperate ϕr_1 /R ₁ C	111
31. Nitrous acid treatment of temperate ϕr_1 /R ₁ C	112
32. Ultraviolet light and nitrous acid survival curves of the clear plaque-forming mutant, ϕr_1 -UV1/R ₁ C	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