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

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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.

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