The Isolation of Non-Excluding Mutants of Phage \( \phi 22 \)

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When bacteria lysogenized by certain temperate phages like \( \lambda \) and \( P2 \) are superinfected by distinguishable mutants of their prophage, the superinfecting phage appears in the progeny released after induction of vegetative phage growth (1, 2). Phage \( \phi 22 \) differs, however, for although bacteria lysogenized with it adsorb superinfecting phage \( \phi 22 \), no superinfecting genetic markers like \( c_2 \) (clear plaque) or \( h \) (host range) appear in the phage obtained after induction, whether spontaneous or produced by u.v. irradiation. Superinfecting phage \( \phi 22 \) is thus excluded and the wild-type prophage may be termed 'excluding' (\( x^+ \)). Non-excluding (\( x \)) mutants have now been isolated by taking advantage of the phenomenon of lysogenic conversion.

\textit{Salmonella typhimurium} only forms somatic antigen 1 (\( O1 \)) if it is infected by phage \( \phi 22 \) possessing the converting gene \( aT^+ \) (3). Non-converting (\( aT \)) mutants exist (4) and when their lysogenic derivatives are superinfected by \( aT^+ \) phage, the bacteria remain \( O1^- \) because the superinfecting \( aT^+ \) gene is excluded (5). This suggested to us that non-excluding mutants of phage \( \phi 22 \) could be isolated by using a strain lysogenized by \( aT^+ \) phage, superinfecting with \( aT^- \) phage, and selecting any bacteria not excluding the superinfecting \( aT^+ \) gene by virtue of their becoming \( O1^+ \). The following procedure proved extremely efficient.

A streptomycin-resistant (\( str-r \)) mutant of \textit{Salmonella typhimurium} strain SR120 (6) was lysogenized by \( aT \ c^+ \) phage kindly provided by P. E. Hartman. An unshaken overnight broth culture was treated with 0.4 M-ethyl methane sulphonate for 20 min. at 37\(^\circ\), and the surviving bacteria grown overnight in broth without shaking at 37\(^\circ\). The overnight culture was then superinfected with \( aT^+ \) phage at a multiplicity of 5 and incubated at 37\(^\circ\) for 30 min., when 0.1 ml. was mixed with 1 ml. of an overnight culture of a \( str-s \) strain of \textit{S. senftenberg}, NCTC5788, and 0.2 ml. of anti-\textit{senftenberg} serum added. Since the somatic antigens of strain SR120 are 4, 5, 12, it is not agglutinated by this serum, which contains anti-1,3,19, unless antigen 1 appears following lysogenic conversion. Any cells of strain SR120 which became \( O1^+ \) would therefore be agglutinated by the serum and would be deposited with the excess of \textit{S. senftenberg} added as carrier. (In \textit{S. senftenberg} antigen 1 does not result from lysogenic conversion (7) and is not subject to 'form variation' (8).) The mixture of bacteria and serum was incubated for 1 hr at 37\(^\circ\), and agglutinated organisms separated by centrifugation for 5 min. at 600 \( g \) in a swing-out head. The deposit was suspended in saline, incubated at 37\(^\circ\) for a further hr, and again centrifuged. The final deposit was added to 100 ml. broth containing streptomycin (1000 \( \mu g./ml. \)) which was incubated overnight and then plated on streptomycin agar to obtain discrete colonies. Each colony was subcultured to 5 ml. broth, incubated overnight without shaking, and infected with \( aT^+ \) phage at a multiplicity of 5. After 60 min. at 37\(^\circ\) each culture was tested by slide agglutination with anti-\textit{senftenberg} serum and examined by low-power dark-field microscopy to detect agglutination.

37

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In the first experiment, 180 clones were tested, the last of which was non-excluding \( (\times) \). Of the other 179 clones, 126 were still \( x^+ \), 35 were \( a^+x^+ \) (presumably back mutants of the \( a^+r \) prophage), and 18 were rough and so agglutinated spontaneously. Selection for \( x \) mutants was therefore improved, as follows. The last two classes of organisms obtained above, being agglutinable before superinfection, were eliminated by treating the cultures with antibody and carrier bacteria and by removing any agglutinated cells before phage was added. Also, the overnight culture in streptomycin broth was streaked out on inositol deoxycholate indicator agar, instead of nutrient agar, containing streptomycin. The difference in colonial appearance between rough and smooth colonies was more pronounced, and any \( str-r \) mutants of \( Salmonella senftenberg \) could be distinguished on sight from \( SR \) because the two strains differ in their ability to ferment inositol. In 8 independent experiments using these modifications, non-excluding mutants were found after testing 12, 19, 2, 10, 26, 4, 8 and 7 clones respectively.

The properties of these mutants have still to be determined in full but it is known that the \( x \) character is indeed a property of the prophage and that all the \( x \) mutants are indistinguishable from \( P 22 \) in morphology, susceptibility to anti-\( P 22 \) serum, heat-resistance, and inability to lyse \( P 22 \)-lysogenic bacteria. Bacteria lysogenized by \( a^+x^+ \) phage develop antigen 01 following superinfection by \( a^+r \) phage only if they come from an unshaken overnight culture and are not growing exponentially when superinfected. The recovery of superinfecting \( c^+h^+a^+r^x^+ \) phage is similarly enhanced by previously starving the bacteria in buffer before superinfection. The expression of superinfecting phage genes is therefore determined by the physiological state of the bacteria at the time of superinfection, and it seems likely that all the non-excluding mutants so far tested are ‘leaky’.

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REFERENCES


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