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Lysogenicity in *Xanthomonas pruni*

BY A. EISENSTARK, S. S. GOLDBERG AND L. B. BERNSTEIN

Department of Bacteriology, Kansas State College, Manhattan, Kansas, U.S.A.

SUMMARY: An apparently lysogenic strain of *Xanthomonas pruni* was isolated. A series of experiments was performed to determine whether the isolate was lysogenic or whether it was a case of pseudolysogenesis. The results indicated that it was a true case of lysogenesis. The phage released by the lysogenic organism differed from the one used in its selection. The possible origin of this new phage and its lysogenic host is discussed.

Several types of *Xanthomonas pruni* phage have been characterized by their differences in plaque morphology, host range, serological properties, in burst times, and in burst sizes (Mandell & Eisenstark, 1952, 1953; Kirchner, 1954). In the process of isolating variant hosts which might be resistant to one or more of the phage types, a culture was obtained and designated as H1 5L, which exhibited the possibility of its harbouring of phage. H1 5L was resistant to all of the *X. pruni* phage types. When dilutions of H1 5L were plated out on a susceptible host, H1, plaques appeared, even though no phage had been added. Such action might have resulted from lysogenesis of H1 5L, or as the result of phage mechanically carried by resistant H1 cells (pseudolysogenesis). These situations have been discussed in detail by Lwoff (1953). A series of tests was performed to determine whether the phenomenon was due to actual lysogenesis existing in *X. pruni* or to carried phage.

METHODS

The procedures for plating, titrating phage and preparing media were essentially the same as those described by Adams (1950) for *Escherichia coli* strain B except that plates were incubated at 24° rather than 37°.

The two *Xanthomonas pruni* hosts described in this report are H1 5L, the suspected lysogenic culture, and H1, obtained originally from the American Type Culture Collection, no. 10,016, which is susceptible to all of the phage types that have been isolated. The types of phage used in the *X. pruni* phage work have been designated as Xp1, Xp2, Xp3, Xp4, Xp5 and Xp8. All of these behave as virulent phages. Xp8 is the phage that is released by host H1 5L. The suspected lysogenic culture, H1 5L, was obtained by plating H1 and an excess of Xp4 phage. After incubation, resistant colonies were selected.

The Xp4 stock used in these experiments was obtained originally by selection of a particular plaque type from a mixed population. In order to minimize the presence of mutant forms, Xp4 stocks for each experiment were prepared by selection of isolated typical Xp4 plaques.

The method described by Adams (1950) was followed in the determination of antiserum inactivation constants.

RESULTS

Isolates of suspected lysogenic organisms were restreaked, incubated, harvested and washed in an attempt to rid them of externally carried phage. This procedure was followed through twenty restreakings. Lysis still occurred when re-isolates were plated back on the susceptible host. When susceptible host H1 was exposed to phage Xp1 and similarly treated, it lost phage Xp1 after the second or third restreaking. This is interpreted as evidence that the phage in the suspected lysogenic culture was not carried mechanically; otherwise it would have been lost upon restreaking as was Xp1.

Antiserum will inactivate extracellular phage but not intracellular. Hence, if culture H1 5L is truly lysogenic, it should be possible to recover phage even though the culture has been transferred through an antiserum medium. It was found that after seven transfers through antiserum medium, culture H1 5L continued to release phage.

Cells of H1 5L were plated on plain nutrient agar to get a regular colony count and on nutrient agar seeded with susceptible host to get a plaque count. In the presence of lysogenesis, there would be an approximate 1:1 ratio of colonies to plaques. If the phage were being carried mechanically, the plaque count should exhibit no relationship to the colony count. The data in Table 1 indicate that the plaques probably originated from single lysogenic organisms, rather than from free phage. Although the ratios are not 1:1, there does appear to be a significant relationship between the number of colonies and the number of plaques.

Table 1. *Ratio of colonies to plaques when aliquots of H1 5L were plated out on (1) nutrient agar and (2) nutrient agar seeded with host H1*

Sample no.	Colonies	Plaques	Ratio
1	302	908	1:3.0
2	75	290	1:3.9
3	37	121	1:3.3

An additional step was to make replica plates (Lederberg & Lederberg, 1952) from colonies of H1 5L on to agar plates which had been seeded with susceptible host. A halo developed around each colony that arose on the new plates, indicating lysis. If this were a consequence of carried phage, the results of this replica plating technique would indicate that each bacterial colony had carried phage, an unlikely situation. A more conceivable explanation is that each colony consisted of lysogenic cells.

A comparison of the phage (Xp8) arising from the lysogenic host with the phage (Xp4) which had been added to the host in the original isolation process revealed distinct differences. If Xp8 had been a carried phage, it should have been identical with Xp4, since Xp4 was the only phage known to have been in contact with this host. Xp8 was found to differ from Xp4 in plaque morphology, as may be seen in Pl. 1 A, B. Almost all of the plaques produced by Xp8 were cloudy, small, and irregular, while Xp4 plaques were much larger,

clear, with a distinct halo. In addition to the cloudy plaques, a small number of larger and clear plaques were produced by Xp8. A few may be seen in Pl. 1B. Studies are in progress to determine the origin and significance of this second plaque type.

Xp8 differs from Xp4 in its host range, as may be seen in Table 2. A series of isolates has been collected which are resistant to one or more of the Xp series of phages. These were used to show that the host range of Xp4 and Xp8 differed.

Table 2. *Host range differences*

Host	Xp4 phage	Xp8 phage
H1	Lysed	Lysed
H1 5L	Not lysed	Not lysed
H80	Lysed	Not lysed
H23	Not lysed	Lysed
H8	Not lysed	Lysed
H204	Not lysed	Lysed

In order to test the possibility that Xp4 stock might contain a small number of contaminant Xp8 phage, Xp4 was plated on host H23, which is resistant to Xp4 but susceptible to Xp8. In the plating of 10^{10} Xp4 phage not a single plaque arose, indicating that if the Xp4 stock was contaminated with Xp8, the contaminant must be present in a frequency less than one out of 10^{10} .

In addition to the above comparisons of Xp4 and Xp8, immunological data were also obtained. Immunologically it was found that Xp4 and Xp8 do not fall into distinct groups, although they are not inactivated at equal rates by Xp4 antiserum. The inactivation constant for the homologous system, Xp4 phage and Xp4 antiserum, was found to be $K=95.6$, whereas the constant for Xp8 phage and Xp4 antiserum was $K=61.5$.

If the phenomenon in question resulted from a carried phage, it should be possible to demonstrate it with any of the Xp phages. H1 5L is resistant to all of the Xp series and if it were merely a selected culture that was carrying phage, it should not make any difference which Xp phage was used in the selection process. However, it was found that Xp4 was the only one of the series that enabled the isolation of H1 5L. In the process of selecting hundreds of resistant organisms, about 25 % of the isolates from Xp4 plates possessed the properties of H1 5L, whereas no isolate from plates seeded with Xp1, Xp2, Xp3 and Xp5 exhibited similar properties.

DISCUSSION

Ordinarily, when dilutions of lysogenic bacteria are plated on a susceptible host, the plaques that arise exhibit a central colony inasmuch as the lysogenic bacterium itself grows into a distinctly visible colony. An occasional organism within a colony bursts and releases phage, hence the plaque around the colony. When H1 5L was plated on H1, central colonies could be seen in only a small percentage of plaques, and then only indistinctly. Possibly the plaque

A. EISENSTARK, S. S. GOLDBERG & L. B. BERNSTEIN—LYSOGENICITY IN
XANTHOMONAS PRUNI. PLATE 1

(Facing p. 405)

itself was so turbid and small that the central colony was obscured by this growth throughout the plaque.

The results of this investigation pose several questions relative to the origin of H1 5L (Xp8). Does H1 actually contain a small number of H1 5L (Xp8) cells, and Xp4 merely select these by lysing the remainder of the population? This seems unlikely in the light of the above experiments where Xp1, Xp2, Xp3 and Xp5 all failed to act as selecting agents.

Are some of the Xp4 phage able to behave as temperate phage that invade H1 cells and cause them to be lysogenized? This would seem to be a possible explanation since lysogenization is a common occurrence among other phage-host systems (Lwoff, 1953). However, if this is the case, why is the phage that is released by H1 5L (Xp8) apparently different from Xp4? A possible explanation, that Xp4 is not pure and contains a small proportion of temperate Xp8 phage capable of lysogenizing host cells, seems unlikely since no plaques arose when 10^{10} Xp4 phage were plated out on H23 which is resistant to Xp4 but susceptible to Xp8. It is also possible that Xp8 represents a host-induced modification (Luria, 1953) of Xp4 which actually has changed its characteristics after a multiplication cycle within its host. A third possible explanation is that the *Xanthomonas pruni* cells harbour Xp8 prophage, but that the mature phage are not developed until the bacterial cells are stimulated to do so by Xp4 nucleic acid. These explanations are in need of careful experimental examination.

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EXPLANATION OF PLATE

- A. Plaques produced by Xp4. These are large and clear with a distinct halo.
- B. Plaques produced by Xp8, the bacteriophage released by lysogenic cells of *Xanthomonas pruni*. These are minute and cloudy. There is always a small percentage of larger plaques. A few of these plaques may be seen in the figure.

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