Xanthomonas phaseoli およびX.begoniae のX.citri テンペレート・ファージによる溶原化,とくに病原性,ファージ感受性および生物学的性質への影響

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者	後藤, 正夫 Starr, M.P.
巻/号	38巻4号
掲載ページ	p. 267-274
発行年月	1972年9月

日植病報 38: 267–274 (1972)
Ann. Phytopath. Soc. Japan 38: 267–274 (1972)

Lysogenization of *Xanthomonas phaseoli* and *X. begoniae* by Temperate *X. citri* Bacteriophages: Effects on Virulence, Phage Sensitivity, and Other Biological Properties

Masao GOTO* and M. P. STARR**

後藤正夫*・M. P. STARR**: *Xanthomonas phaseoli* および *X. begoniae* の *X. citri* テンペレート・ファージによる溶原化，とくに病原性，ファージ感受性および生物学的性質への影響

Abstract

When temperate phages of *Xanthomonas citri* infected other susceptible xanthomonads, these bacteria (*X. begoniae and X. phaseoli*) were lysogenized and released temperate phages constantly thereafter. The lysogenized bacteria did not lose their lysogenicity after repeated transfer through their original host plants. The lysogenized bacteria sometimes showed great changes in susceptibility to either the temperate phages or the virulent phages. However, no change was noticed in other characteristics, including plant pathogenicity and utilization of carbon sources, indicating that these temperate phages of *X. citri* did not cause transductions of these characters in these bacteria under the conditions used.

(Received July 13, 1971)

Lysogenicity has been demonstrated among the phytopathogenic bacteria in a number of species, including *Xanthomonas pruni*[2], *X. malvacearum*[6], *X. citri*[8], *Pseudomonas solanacearum*[7], *P. syringae* and *P. morsprunorum*[3], *Corynebacterium sepedonicum*[10] and *Agrobacterium tumefaciens*[1,11].

Many isolates of *Xanthomonas citri* have been reported[8] to be lysogenic, releasing temperate phages with various host range patterns. We have shown[5] that, among 136 isolates of 43 nomenspecies of the genus *Xanthomonas*, only six isolates (*X. begoniae* XB8 and XB111, *X. cucurbitae* XC6, *X. phaseoli* XP4 and Xph, and *X. translucens* f. sp. *phleum-pratense* XT103) showed susceptibility against any of the six temperate *X. citri* bacteriophages used. *X. begoniae* XB8 and *X. phaseoli* XP4 were particularly interesting to us in view of their broad susceptibility to most of those phages. Therefore, experiments were conducted with several aims including a determination of whether or not lysogenization of these bacteria with these temperate *X. citri* phages might induce significant changes in the characteristics of the recipient bacteria, and thus provide some evidence for the occurrence of transduction in the xanthomonads.

Materials and methods

Bacterial cultures The histories and phage-host relationships of the bacterial cultures used in this study are given in our previous paper[5]. All cultures were maintained on potato-glucose or

* Faculty of Agriculture, Shizuoka University, Iwata, Shizuoka, Japan. 静岡大学農学部
** Department of Bacteriology, University of California, Davis, California, U.S.A. カリフォルニア大学

yeast-glucose-$CaCO_3$ agar slants, and stored at 5°C during this investigation.

Culture media, phage preparations, and phage susceptibility tests The materials and methods presented in our previous report[5] were, in general, utilized here. The histories of the phage preparations which we have used are recorded in Table 1 of the paper[5] by Goto and Starr (1972). These temperate phages had been isolated from naturally lysogenized *X. citri* cultures.

Lysogenization Phage suspensions used for lysogenization were confirmed to be free from live bacterial cells by streaking a drop of each on the surface of nutrient agar plates, or by incubating the phage preparations themselves at 28°C for 2 days. When there was no bacterial growth at all on the plates and in the phage preparations, the phage samples were judged to be free from any bacterial cells. A drop of each phage preparation was put on the surface of agar plates which had been inoculated with the cells of a susceptible indicator bacterium. After 24 hours of incubation, a small piece of agar was cut from the central area of turbid plaques produced by the phages, transferred to test tubes of peptone-sucrose solution, and incubated at 28°C for 1 to 2 days. When a slight bacterial growth occurred in the test tubes, the preparation was diluted properly and plated. After 48 hours the mixtures of 2 m*l* of soft agar and 0.5 m*l* of the culture of the susceptible bacteria were poured into the plates so that the thin film of the soft agar was spread over the well separated bacterial colonies. Spreading the soft agar over the bacterial growths was carried out very gently, without shaking, so that the colonies retained their original shape. After 24 hours of incubation at 28°C, clear zones were seen around some colonies. These colonies were diluted again in peptone water and plated on agar medium to purify the clones as well as to determine whether the clear zones around colonies appeared as the results of lysogenization or possibly because of carrier phages. These plates were poured again with soft agar mixed with the susceptible bacteria. These procedures were repeated three to five times. When 100 percent of the colonies on plates showed in succession the formation of clear zones around them, it was judged that the clones were lysogenized.

Plant pathogenicity The plants used for pathogenicity tests were grown in a greenhouse. They were inoculated by puncturing the surfaces of the leaves with a fine sharp needle through droplets of bacterial suspensions. After inoculation, the plants were kept under moist conditions by covering them with polyethylene bags for 24 hours. Bean seedlings (variety: Shin-Edogawa) and young Natsudaidai seedlings were used as the host plants for inoculation. In addition to the method described above, the following method was also applied: the bacterial cell suspensions were injected with a syringe into the mesophyll of the leaves and left in the greenhouse. In all cases, the parent bacteria, the lysogenic bacteria and the susceptible recipient bacteria, were inoculated as controls in parallel with the artificially lysogenized clones.

Bacteriological characteristics of lysogenized bacteria The procedures in the Manual of Microbiological Methods[12] (Society of American Bacteriologists 1957) were followed for determining bacteriological characteristics.

Results and discussion

Lysogenization of some xanthomonads with *X. citri* temperate phages and characteristics of the lysogenized bacteria

The plaques that formed on the indicator bacteria were transferred to peptone-sucrose solution, and incubated for 24 hours at 28°C. In the first platings from such cultures, 20 to 90 percent of the colonies (depending on the phages used) seemed to be lysogenized, as evidenced by the formation of clear plaques around the colonies. However, on the second series of plates, on which the afore-

Table 1. Change in phage reactions[a] of *Xanthomonas phaseoli* through lysogenization by *X. citri* temperate phages (N64p, U85p, N201p, and N200p)[5]

Bacteria (Lysogenized by)		Phage preparations[b]															
		Sp3	ph1	ph7	ph11	ph22	ph25	ph219	XM106	Cp1	Cp2	N64p	U7p	49-1bp	U85p	U104p	U76p
XP4 (N64p)	1[c]	4+	4+	−	4+	4+	−	4+	−	−	4+	−	3+	3+	−	3+	2+s
	2	4+	4+	−	4+	4+	−	4+	−	−	−	−	−	−	−	−	−
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XP4 (U85p)	1	4+	4+	−	4+	4+	−	4+	−	−	4+	3+	3+	3+	−	3+	−
	2	4+	4+	−	2+	2+	−	2+	−	−	4+	3+	−	3+	−	3+	−
	3	4+	4+	−	2+	2+	−	2+	−	−	−	−	−	−	−	−	−
XP4 (N201p)	1	4+	4+	−	4+	4+	−	4+	−	−	4+	2+	3+	3+	−	3+	−
	2	4+	4+	−	4+	4+	−	4+	2+s	−	4+	3+	3+	3+	−	3+	−
	3	4+	4+	−	4+	4+	−	3+	−	−	−	−	−	2+	−	−	−
	4	4+	4+	−	4+	4+	−	−	−	−	−	−	−	−	−	−	−
	5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XP4 (N200p)	1	4+	4+	−	3+	4+	−	4+	−	−	4+	3+	3+	3+	−	3+	−
	2	4+	3+	−	3+	3+	−	3+	−	−	1+	3+	3+	3+	−	3+	−
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XP4 parent		4+	4+	−	4+	4+	−	4+	2+	−	4+	3+	3+	3+	3+	3+	−
U85 parent		−	−	−	−	−	2+	−	−	2+	−	−	−	−	−	−	−
N201 parent		4+	3+	−	4+	4+	2+	3+	−	4+	−	3+	−	2+	−	2+	−
N200 parent		4+	3+	−	4+	4+	3+	3+	−	4+	−	3+	2+	2+	−	3+	−
N64 parent		−	−	−	−	−	2+	−	−	−	−	−	−	−	−	−	−

a) Symbols used: 4+, clear plaques with no trace of turbidity; 3+, plaques with a trace of turbidity; 2+, plaques with a moderate turbidity; 1+, very turbid plaques; −, no plaques; s, isolated, scattered plaques.

b) See Goto and Starr (1972) for histories and characteristics of these phage preparations[5].

c) The numerals in this vertical column indicate lysogenized clones differing in phage susceptibility patterns.

Table 2. Change in phage reactions of *Xanthomonas begoniae* through lysogenization by *X. citri* temperate phages (49–16p, U76p, N64p and U7p)[5)]

Bacteria (Lysogenized by)		Phage preparations														
		Sp3	ph1	ph7	ph11	ph22	ph25	ph219	XM106	Cp1	Cp2	N64p	U7p	49–1bp	U85p	U104p	U76p
XB8 (49–1bp)	1	−	2+	−	−	3+	2+	−	−	−	−	−	−	−	−	−	−
	2	−	2+	2+	−	3+	2+	−	−	−	−	−	−	−	−	−	−
	3	−	3+	2+	2+	3+	2+	−	−	−	−	−	−	−	−	−	−
XB8 (U76p)	1	−	2+	2+	2+	2+	3+	3+	−	−	−	2+	−	−	−	−	−
	2	−	4+	4+	4+	4+	3+	4+	−	−	−	−	−	−	−	−	−
XB8 (N64p)	1	−	4+	4+	4+	4+	3+	4+	−	−	−	−	−	−	−	−	−
	2	−	4+	4+	4+	4+	3+	4+	−	−	−	−	−	2+	−	−	−
XB8 (U7p)	1	−	−	3+	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XB8 parent		−	4+	4+	4+	4+	3+	4+	−	−	−	2+	3+	2+	2+	−	2+
49–1b parent		−	−	−	−	−	2+	−	−	−	−	−	−	−	−	−	−
U76 parent		−	−	−	−	−	2+	3+	−	−	−	−	−	−	−	−	−
N64 parent		−	−	−	−	−	2+	−	−	−	−	−	−	−	−	−	−
U7 parent		−	−	−	−	−	2+	−	−	−	−	−	−	−	−	−	−

Symbols and conventions: same as in Table 1.

mentioned seemingly lysogenized colonies were plated again, 100 percent of the colonies produced the distinctive plaques around them. This could be successively reproduced repeated even after the repeated inoculation reisolation processes through the host plant, indicating that these plaques were formed by phages which originated in the lysogenized bacteria and not in the carrier strains. The isolates of *X. phaseoli* (XP4) and *X. begoniae* (XB8) could be lysogeinzed successfully with the temperate *X. citri* phages N200p, N201p, N64p, U85p, 49-1bp, U76p and U7p.

The lysogenized bacteria showed great variation in their susceptibility patterns, not only against the temperate phages but also against some virulent phages (Tables 1 and 2). The newly lysogenized clones became immune to the temperate phages used for lysogenization. When *X. phaseoli* (XP4) cells were used as the recipient bacteria, the strains lysogenized with any of the phages always became resistant to phage U85p (Table 1). Since some cultures of isolate XP4 lysogenized with temperate phage U85p still had susceptibility to other temperate phages, and the reverse was also true except the reactions to the phage U85p, these phages might be considered to be genetically different from each other. In this case, there might also be the possibility that these isolates (N64, N200, and N201) are doubly-lysogenic bacteria which release phage U85p in common in addition to their own particular phages, whereas isolate U85 is a singly-lysogenic strain producing only phage U85p. In the isolate of *X. begoniae* (XB8), however, there was a discrepancy, namely, that susceptibility to the other temperate phages was almost lost through lysogenization (Table 2). Further, the lysogenization made the bacteria resistant also to some virulent phages. Thus, the pattern of phage-susceptibility of lysogenized bacteria seems to result from complicated interactions between prophages and bacteria. This situation is left as an open question for future study.

Although the phage susceptibility patterns changed to various degrees through lysogenization, the basic patterns of the lysogenized strains were generally those of the recipient strains. Isolates XP4 and XB8 did not take in, through lysogenization, the susceptibility to certain phages which was found in the donor strains but not in the recipient. Therefore, transduction of the donor genes controlling the phage susceptibility into the recipient cells seems not to have occurred. Thus, it is suggested that this lysogenization, as well as mutation, might have important roles in nature for the distribution of the various phage susceptibility patterns[5,9] within a *Xanthomonas* nomenspecies.

When *X. phaseoli* (XP4) was lysogenized with the temperate phage N201, the second progenies, which were derived from a single colony culture with the type *a* pattern, were divided into two phage susceptibility types as shown in Table 3. Type *b* was resistant to all phages tested. In the third progenies, however, five different patterns were recognized including type *b* in the second progenies as well as the pattern which closely resembled that of type *a*. The other patterns (*c*, *d*, and *e*) seemed to be intermediates between these two. The progenies from type *b* were quite uniform and showed no further variation. These results indicated that the phage-susceptibility of the lysogenized bacteria may be variable and that the variation proceeded toward clones resistant to all available phages.

It was noticed, sometimes, that some of the lysogenized bacteria (but not the unlysogenized recipient bacteria) formed many tiny plaques on their growth on the plates. When the plaques were tested on other lysogenized strains, it was confirmed that the phage greatly broadened its host range, suggesting that the phage changed its infectivity through mutation or some other mechanisms during the lysogenization. This fact suggested that the temperate phages can be altered in their infectivity through lysogenization even though the lysogenized bacterium was still immune to the phages. The single colonies of the lysogenized bacterium XP4 (U85) were grown in peptone-sucrose solution and the infectivity of their phages tested by streaking (after filtration) on each

Table 3. Variation of the phage susceptibility through serial single colony cultures in *Xanthomonas phaseoli* (XP4) which was lysogenized with temperate phage N201p

Bacteria	Phage preparations												
	Sp3	ph1	ph11	ph22	ph219	Cp2	N64p	U7p	49-1bp	U85p	U76p	U104p	Pattern
XP4 parent	4+	4+	4+	4+	4+	4+	3+	3+	3+	3+	3+s	3+	
1st, 7d	4+	4+	4+	4+	4+	4+	3+	3+	3+	−	−	3+	*a*
2nd, 7d-1	4+	4+	4+	4+	4+	4+	3+	3+	3+	−	−	3+	*a*
7d-2	−	−	−	−	−	−	−	−	−	−	−	−	*b*
3rd, 7d-1-1	4+	4+	4+	4+	4+	4+	3+	3+	3+	−	2+s	3+	*a*
7d-1-2	3+	3+	3+	2+	3+	−	−	3+	3+	−	−	3+	*c*
7d-1-3	2+	3+	−	2+	3+	−	−	−	3+	−	−	−	*d*
7d-1-4	2+	−	−	2+	−	−	−	−	3+	−	−	−	*e*
7d-1-5	−	−	−	−	−	−	−	−	−	−	−	−	*b*
7d-2-1	−	−	−	−	−	−	−	−	−	−	−	−	*b*

Symbols and conventions: same as in Table 1.

culture. The results are shown in Table 4. Every clone tested proved to be lysogenized. However, the phages produced by them showed quite different behaviors on the other lysogenic clones. The phages produced by clones 4, 6 and 7 were analogous to the original phage, U85p. The phages from clones 1, 2, 3 and 5 were different from the former in that they had broader host ranges including action on some of the lysogenic clones.

It is remarkable that the infectivity of the phages established in the artificially lysogenized strains, as well as the phage-susceptibility of the lysogenized bacteria, were extremely variable. Although similar behavior sometimes could be observed in the original isolates of *X. citri*[4], another question left for future answer is the frequency of the occurrence of such phenomena among the isolates of *X. citri*.

Table 4. Variation of the temperate phages through lysogenization as shown by the typing patterns

Bacteria (Lysogenized by)	Phage preparations							
	U85p	XP4(U85p)-1	-2	-3	-4	-5	-6	-7
XP4	3+s	3+	3+	3+	3+	4+	4+	3+s
U85	−	−	−	−	−	−	−	−
XP4 (U85p)-1	−	−	3+	3+	−	3+	−	−
XP4 (U85p)-2	−	−	−	−	−	3+	−	−
XP4 (U85p)-3	−	−	−	−	−	3+	−	−
XP4 (U85p)-4	−	3+s	3+	3+	−	4+	−	−
XP4 (U85p)-5	−	−	−	−	−	−	−	−
XP4 (U85p)-6	−	3+s	3+	3+	−	4+	−	−
XP4 (U85p)-7	−	3+s	3+	3+	−	4+	−	−

Symbols and conventions: same as in Table 1.

Plant pathogenicity of lysogenized bacteria

X. phaseoli (XP4) lysogenized, respectively, with temperate phages N64p, U85p, N200p, N201p, U76p, and 49-1p were inoculated on the leaves of bean and on Natsudaidai orange by the wound inoculation method. As a control, the isolates of *X. phaseoli* (XP4) and *X. citri* (N64, U85, N200, N201, U76 and 49-1b) were inoculated as well. The isolates of *X. phaseoli* (XP4) and all lysogenized bacteria produced on bean leaves water-soaked lesions around punctures after a few days and the lesions soon turned reddish brown. A wide yellow halo appeared around the lesions in 10 to 14 days after inoculation and developed into the typical symptoms of bacterial blight of bean. No isolate of *X. citri*, however, caused any symptoms on the inoculated bean leaves but did induce hypersensitivity reactions when infiltrated at high inoculum doses. On the other hand, no lysogenized bacteria, nor the parent *X. phaseoli* (XP4), showed pathogenicity on citrus leaves by wound inoculation. All isolates of *X. citri* produced typical citrus canker lesions through the same procedures. When the bacterial suspension of the lysogenized strains was infiltrated into mesophyll of the citrus leaves, the tissues showed some swelling with somewhat water-soaked appearance in the margins, and later yellowed. The swollen tissues, however, never developed into typical frank cankers, so that the reaction was not considered as a real symptom of the citrus canker disease. Thus, it might be concluded that lysogenization of *X. phaseoli* with the temperate phages of *X. citri* did not introduce the slightest shift toward pathogenicity on citrus in the recipient bacteria under the present experimental conditions.

Bacteriological characteristics of lysogenized bacteria

Carbon source utilization among the isolates of *X. phaseoli*, *X. begoniae*, and *X. citri* was tested using xylose, arabinose, rhamnose, glucose, fructose, galactose, mannose, saccharose, maltose, lactose, cellobiose, dextrin, starch, inulin, glycerol, adonitol, dulcitol, mannitol, sorbitol, inositol, salicin, succinic acid, citric acid, malic acid, and tartaric acid. Differences were found only in the ability to oxidize lactose, maltose, salicin, sorbitol, and glycerol. The isolates of *X. phaseoli* (XP4) and *X. begoniae* (XB8) were lysogenized with the phages derived from the isolates of *X. citri* which differed from XP4 and XP8 in the ability to utilize these substances. However, the properties of the newly lysogenized bacteria were just the same as those of recipient bacteria and did not change at all.

Thus, when the strains of *X. phaseoli* and *X. begoniae* were lysogenized with the temperate phages of *X. citri*, only the phage susceptibility changed—shifting toward resistance to any other phages. Although the dissociation of the resistant clones to which no *X. citri* phages showed infectivity could be found in the cultures of XP4 and XB8 without the phage effects, the frequency of the appearance of the resistant clones in this case was much lower than that in the lysogenized bacteria. The shift toward phage-resistance in these bacteria, therefore, seems to be determined by the prophages.

Acknowledgements

Supported by a research grant (AI-08426) from the National Institute of Allergy and Infectious Diseases and by an International Postdoctoral Fellowship (TW-01031), both awarded by the United States Public Health Service. We are grateful to Dr. Arun K. Chatterjee for critical readings of the manuscript.

Literature cited

1. Beardsley, R. E. (1960). J. Bact. 80, 180–187.
2. Eisenstark, A., and Bernstein, L. B. (1955). Phytopathology 45, 596–598.
3. Garrett, C. M. E. and Crosse, J. E. (1963). J. Appl. Bact. 26, 27–34.
4. Goto, M. (1970). Ann. Phytopath. Soc. Japan 36, 171. (Abstract)
5. Goto, M. and Starr, M. P. (1972). *Ibid.* 38, 226–248.
6. Hayward, A. C. (1964). J. Gen. Microbiol. 35, 287–298.
7. Okabe, N. (1954). Bull. Fac. Agr. Shizuoka Univ. 4, 28–36.
8. Okabe, N. (1961). *In* Commemoration of Dr. T. Matsumoto pp. 61–73.
9. Stolp, H. and Starr, M. P. (1964). Phytopath. Zeits. 51, 442–478.
10. Trofimets, L. N. and Shneider, Y. I. (1969). Biol. Nauki, 12, 96–100. (Rev. Plant Path. 49, 166).
11. Zimmerer, R. P., Hamilton, R. H. and Pootjes, C. (1966). J. Bact. 92, 746–750.
12. Society of American Bacteriologists, Committee on Bacteriological Technic (1957). Manual of Microbiological Methods, McGraw-Hill, New York.

和文摘要

Xanthomonas phaseoli および *X. begoniae* の *X. citri* テンペレート・ファージによる溶原化，とくに病原性，ファージ感受性および生物学的性質への影響

後藤正夫・M. P. STARR

Xanthomonas phaseoli および *X. begoniae* に *X. citri* のテンペレート・ファージを感染させると，これらの細菌は溶原化され，同じテンペレート・ファージを放出した．溶原化された細菌は寄主植物を通過してもその溶原性を失わなかった。これらの細菌の他のファージに対する感受性には，溶原化によって大きな変化が起る場合が多かったが，病原性および炭水化物分解性には何ら変化は認められなかった。一度溶原化された細菌も，培養中に，他のファージに対する感受性を異にしたクローンを生ずることが単集落培養によって明らかになった。同様に寄主範囲を異にするファージを放出するクローンの出現も認められた。