

Xanthomonas campestris pv. citriの産生する繊維状ファージ

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Filamentous Phages Released from *Xanthomonas campestris* pv. *citri*

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上運天 博*・古場潤一*・脇本 哲* : *Xanthomonas campestris* pv. *citri* の産生する繊維状ファージ

Abstract

Two kinds of filamentous phage, named XCf1, and XCf2, were found to be released from the living cells of *Xanthomonas campestris* pv. *citri* isolates and some of their properties were investigated in comparison with *X. campestris* pv. *oryzae* phages Xf and Xf2.

The phages XCf1 and XCf2 were released from the bacterial isolates N 6101-1 and N 6107, respectively and they could attack only *X. campestris* pv. *citri* isolates. They multiplied in a liquid medium in parallel with bacterial multiplication to reach maximum concentration at about 10^5 PFU/ml. The phages XCf1 and XCf2 were similar in particle length ranging from 800 to 1100 nm. Both phages were heat resistant and survived even the treatment at 80 C for 10 min, but inactivated by the treatment with organic solvents such as chloroform, methyl alcohol, ethylether and acetone.

Serological inactivation tests revealed that both phages were closely related each other. Anti-XCf1 serum readily inactivated the phage Xf, but anti-Xf serum did not inactivate the phage XCf1, suggesting some relationship between the two. The phage XCf2 showed very slight serological relationship with Xf but not at all with Xf2.

Ten isolates of *X. campestris* pv. *citri* out of 33 tested released XCf1 and 4 released XCf2.

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Introduction

The filamentous phages of *X. campestris* pv. *oryzae* were isolated, purified and characterized in the previous papers^{4,5}. A filamentous phage from *X. campestris* pv. *citri* was first isolated by Dai *et al.*^{2,3} and some of its properties were reported. In this experiment, we also successfully isolated filamentous phages from Japanese isolates of *X. campestris* pv. *citri* and some of their properties were clarified. Since *X. campestris* pv. *citri* has close relationship with *X. campestris* pv. *oryzae* in many bacteriological characteristics, the serological relationships among filamentous phages released from both pathovars were investigated.

Materials, Methods and Results

Bacteria and phages

All the isolates of *X. campestris* pv. *citri* except Ku7103 and other xanthomonads used in this experiment were furnished by National Institute of Agricultural Sciences, Tsukuba Science City, Japan. Ku7103 was isolated from diseased citrus leaf in this laboratory.

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Media

PS medium was used for the purpose of growing bacteria and propagating of phages, the composition of which was: decoction of 300 g of potato; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2 g; $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.5 g; peptone, 5 g; sucrose, 20 g; water 1 liter, pH 7.0. PS medium fortified with 0.6 % agar was used for plaque formation and that with 1.5 % for colony formation.

Isolation of filamentous phages

The method applied for the isolation of filamentous phage was as described in the previous papers^{4,5}.

The filamentous phages named XCf1 and XCf2 were isolated respectively from the culture fluids of *X. campestris* pv. *citri* isolates N 6101-1 and N 6107. The isolates of *X. campestris* pv. *citri* N 6112-a and N 6125-1 were used as indicators for assaying respective filamentous phages XCf1 and XCf2, because the centrifuged supernatant of culture suspension of these bacteria did not produce plaque on any isolates of *X. campestris* pv. *citri*. Both phages produced tiny plaques on the lawn of indicator bacteria as shown in Fig. 1.

Concentration of the filamentous phages in bacterial cultures

Single colony of *X. campestris* pv. *citri* isolate N 6107 or N 6101-1 on PSA medium was transferred to PS liquid medium and cultured on a shaker overnight at 30 C. The culture fluid was centrifuged at $8,000 \times g$ for 15 min to precipitate bacterial cells. The precipitant was resuspended in fresh PS medium and cultured by shaking at 30 C for 36 hr. Periodically, 0.5 ml of the culture fluid was taken out, 0.1 ml of which was used for counting bacterial population and the rest was used for plaque counting after discarding bacterial cells by centrifugation. The plates were incubated for 24 hr for counting plaques and 72 hr for counting colonies after plating.

Both bacterial isolates N 6107 and N 6101-1 multiplied to reach maximum concentration

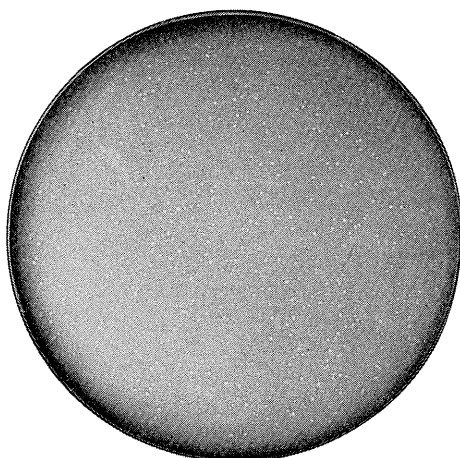


Fig. 1. Plaques produced by *X. campestris* pv. *citri* filamentous phage XCf1 on the lawn of *X. campestris* pv. *citri* N 6112-a

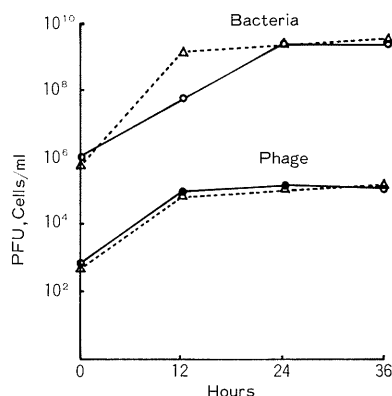


Fig. 2. Bacterial growth and liberation of filamentous phages in culture suspension.

(—○—): *X. campestris* pv. *citri* N 6101-1 and its phage XCf1

(---△---): *X. campestris* pv. *citri* N 6107 and its phage XCf2

of about 10^9 cells/ml and they released respective phages XCf1 and XCf2 at the concentration of about 10^5 PFU/ml (Fig. 2).

Host range of the filamentous phages

The host range of the filamentous phages XCf1 and XCf2 was tested by plaque formation method. One ml each of the bacterial suspension to be tested was added with 0.01 to 0.5 ml of the adequate dilution of the suspension of phage XCf1 or XCf2. Nine ml of PSA medium containing 0.6 % agar, melted and kept at 50 C, was added, and poured into petri dishes. The plate was incubated at 30 C for 48 hr.

As shown in Table 1, *X. campestris* pv. *citri* isolate N6112-a was susceptible to the filamentous phage XCf1 released from N6101-1 but resistant to the phage XCf2 released from N6107. The isolate Ku7103, however, showed opposite result. Other isolates of *X. campestris* pv. *citri* tested were susceptible, while all of the other xanthomonads tested were resistant to both phages.

Inactivation of the filamentous phages by heat treatment and organic solvents

Small test tubes containing 0.5 ml of the phage suspension (about 10^9 PFU/ml) were dipped in a water bath the temperature of which was adjusted from 50 to 90 C at 10 C intervals. After 10 min of incubation, phage activity was assayed. To test the effect of organic solvents, 0.5 ml of the phage suspension was added to an equal volume of each solvent, shaken at 30 C for 30 min, and the phage survived in aqueous layer was assayed by plaque count method. Since methyl alcohol and acetone were soluble in water, the mixture was taken out for plaque counting and values obtained were doubled.

Both phages could survive even after the heat treatment at 80 C for 10 min, but they were completely killed at 90 C for 10 min (Fig. 3).

With regard to the effect of organic solvents, all of the 4 organic solvents used in this experiment inactivated both phages, and the effects of acetone and chloroform were much higher than those of others as shown in Table 2.

Table 1. Host range of the filamentous phages XCf1 and XCf2 respectively released from *X. campestris* pv. *citri* N6101-1 and N6107

Bacteria	Phage	XCf1	XCf2
<i>Xanthomonas campestris</i> pv. <i>citri</i>			
	N6112-a	+	-
	N6114-1	+	+
	N6125-1	+	+
	N6130	+	+
	Ku7103	-	+
<i>X. campestris</i> pv. <i>oryzae</i>			
	N5808	-	-
	N5845	-	-
	N5850	-	-
	N5854	-	-
	N5859	-	-
	H5809	-	-
	B-69	-	-
<i>X. campestris</i> pv. <i>campestris</i>		-	-
<i>X. campestris</i> pv. <i>cucurbitae</i>		-	-
<i>X. campestris</i> pv. <i>hyacinthi</i>		-	-
<i>X. campestris</i> pv. <i>phaseoli</i>		-	-
<i>X. campestris</i> pv. <i>physalidicola</i>		-	-
<i>X. campestris</i> pv. <i>psi</i>		-	-
<i>X. campestris</i> pv. <i>pruni</i>		-	-
<i>X. campestris</i> pv. <i>hordei</i>		-	-
<i>X. campestris</i> pv. <i>vesicatoria</i>		-	-
<i>X. campestris</i> pv. <i>vitians</i>		-	-

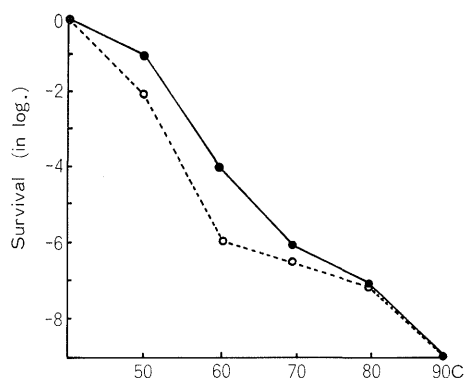


Fig. 3. Inactivation of filamentous phages by heat treatment at respective temperatures for ten minutes.

(—●—): XCf1 (---○---): XCf2

Table 2. Stability of the filamentous phages XCf1 and XCf2 in organic solvents

Treatment \ Phage	XCf1	XCf2
	PFU/ml	PFU/ml
None	3.9×10^9	1.2×10^9
Methyl alcohol	9.8×10^8	4.0×10^8
Ethylether	3.3×10^6	1.4×10^5
Acetone	0	0
Chloroform	0	0

Morphology of the filamentous phages

Both phages XCf1 and XCf2 were purified by the same method as in the case of Xf⁽⁴⁾ and Xf⁽⁵⁾, the filamentous phages of *X. campestris* pv. *oryzae*. The purified preparations obtained by sucrose density-gradient centrifugation were mounted on carbon coated grids, and stained with 2 % uranyl acetate solution. Observations were carried out with JEM-100 S electron microscope (Fig. 4).

From the length distribution histograms shown in Fig. 5, it will be concluded that the lengths of the particles of both phages XCf1 and XCf2 are almost similar, ranging from 800 to 1,100 nm.

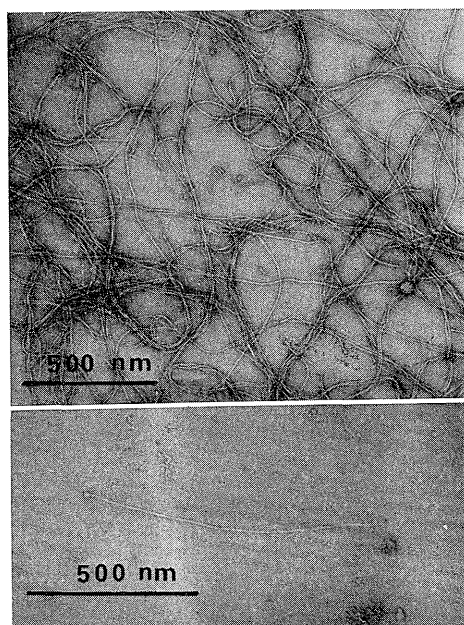


Fig. 4. Electron micrographs of the purified XCf1 phage.

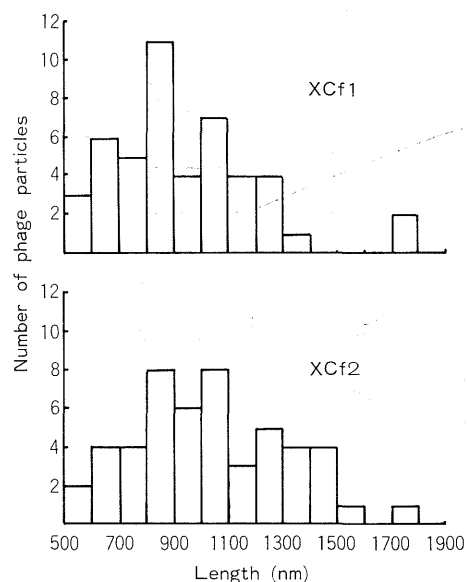


Fig. 5. Length distribution histograms of filamentous phages XCf1 and XCf2.

Serological relationship of the filamentous phages

The anti-phage sera were prepared by injecting partially purified phage suspensions of XCf1, XCf2, Xf and Xf2 to rabbits 6–7 times at 4 days interval. Seven days after the final injection, antisera were obtained by ordinary procedures. The cross-neutralization test was conducted according to Adams' methods¹⁾. The results are shown by K values in Table 3. The antisera prepared against the phage XCf1 neutralized not only both phages XCf1 and XCf2 but also Xf released from *X. campestris* pv. *oryzae* as well. Neither anti-XCf1 nor anti-XCf2 serum, however, inactivated another filamentous phage Xf2 of *X. campestris* pv. *oryzae*. The anti-XCf2 serum neutralized both phages XCf1 and XCf2 and slightly inactivated the phage Xf. Anti-Xf serum could inactivate XCf2 very slightly but not XCf1 at all.

These results of serological tests suggest that two phages XCf1 and XCf2 were closely related each other and they had a relationship with phage Xf to some extent, but not with Xf2.

Distinction of phages released from X. campestris pv. citri isolates

The phages released in the culture fluid from many isolates of *X. campestris* pv. *citri* were detected by using *X. campestris* pv. *citri* isolates N 6112-a, N 6114-1, N 6125-1, N 6103 and Ku 7103 as indicators. If the plaques appeared on the plate of indicator bacteria, single plaque isolation was carried out and was enriched by inoculating to the susceptible bacteria. The distinction of the phages thus propagated was made in terms of host range and serological properties. The methods applied for these experiments were as the same described above.

As shown in Table 4, 10 bacterial isolates out of 33 tested released the phages similar to XCf1 in host range and 4 released the phages similar to XCf2. All phages released from *X. campestris* pv. *citri* were inactivated by both of XCf1 and XCf2 antisera.

Table 3. Serological relationships among filamentous phages of *X. campestris* pv. *citri* and pv. *oryzae*

Anti-phage serum \ Phage	<i>X. campestris</i> pv. <i>citri</i> phage		<i>X. campestris</i> pv. <i>oryzae</i> phage	
	XCf1	XCf2	Xf	Xf2
XCf1	1637 ^{a)}	162	1118	0
XCf2	144	173	9	0
Xf	0	5	1482	0
Xf2	0	0	0	67

a) K value

Table 4. Frequency of *X. campestris* pv. *citri* isolates releasing the filamentous phages XCf1 and XCf2

Number of <i>X. campestris</i> pv. <i>citri</i> isolates tested	Total number of bacterial isolates released XCf1 or XCf2	Number of bacterial isolates released XCf1	Number of bacterial isolates released XCf2
33	14	10	4

Discussion

Two kinds of filamentous phage, XCf1 and XCf2, released from *X. campestris* pv. *citri*, could not infect any xanthomonads other than *X. campestris* pv. *citri*. When the phage-producing bacteria were cultured shaking in liquid medium, the concentration of these phages reached to 10^5 PFU/ml with bacterial growth. This concentration was higher than that of the filamentous phages of *X. campestris* pv. *oryzae*, Xf and Xf2^{4,5)}.

No remarkable difference was observed between the phage XCf1 and XCf2 in response to the treatments by heat and organic solvents. Even by the heat treatment at 80 C for 10 min, some of both phages survived. Both of them were susceptible to organic solvents as in the case of Xf and Xf2.

Dai *et al.*²⁾ isolated a filamentous phage from an isolate of *X. campestris* pv. *citri* in Taiwan and designated as Cf. They reported that Cf and Xf were closely related each other in serological properties. Two phages XCf1 and XCf2 isolated from Japanese isolates of *X. campestris* pv. *citri* were closely related each other in serological neutralization test. However, serological relationship of these phages to Xf was not complete, *i. e.*, anti-XCf1 serum inactivated Xf but anti-Xf serum did not XCf1. In the same serological tests with the phages XCf2 and Xf and their antisera, K values were very low in heterologous combinations in comparison with those of homologous combinations. Both XCf1 and XCf2 were serologically different from Xf2.

These results suggest that the phages XCf1 and XCf2 isolated in our experiment seem to be different from Cf reported by Dai *et al.*²⁾.

Among 33 isolates of *X. campestris* pv. *citri* tested, 14 produced filamentous phages. All of these phages were inactivated by both anti-XCf1 and anti-XCf2 sera, suggesting that they belonged to either XCf1 and XCf2. By testing host range of these phages, it was clarified that 10 isolates released XCf1 and the other 4 released XCf2.

Literature cited

1. Adams, M. H. (1959). Bacteriophages. Interscience publishers, Inc., New York.
2. Dai, H., Chiang, K-S. and Kuo, T. T. (1976). Festschrift in Memorial President Chiang Kai-Shek. Academia Sinica, Taipei, Republic of China. pp. 411-426.
3. Dai, H., Chiang, K-S. and Kuo, T. T. (1980). J. gen. Virol. 46: 277-289.
4. Wakimoto, S., Katsuki, Y., Matsuo, N. and Kamiunten, H. (1979). Ann. Phytopath. Soc. Japan 45: 168-173.
5. Kamiunten, H. and Wakimoto, S. (1979). Ann. Phytopath. Soc. Japan 45: 174-181.

和文摘要

Xanthomonas campestris pv. *citri* の産生する繊維状ファージ

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Xanthomonas campestris pv. *citri* から放出されている二種類の繊維状ファージに対して XCf1 と XCf2 と命名し、それらの生物学的な諸性質を *X. campestris* pv. *oryzae* のファージ Xf および Xf2 と比較しながら検討した。ファージ XCf1 および XCf2 はそれぞれ分離株 N6101-1 および N6107 から放出され、培養液中の最高濃度はともに約 10^8 PFU/ml に達した。これらのファージは *X. campestris* pv. *citri* のみを侵すことができ、実験に供した他の xanthomonads 属菌は侵すことができなかった。両ファージの粒子の長さはほぼ同じで 800~1,100nm であった。両ファージは熱に対して比較的安定で、80°C, 10分間処理した後もなお活性を維持していた。しかし、クロロホルム, メチルアルコール, エチルエーテル, アセトン等の有機溶媒によって不活化された。両ファージの抗血清は相互に中和反応を示し、血清学的に密接な関係にあることが明らかになった。また、抗 XCf1 血清は Xf を容易に不活化した。しかし、逆に抗 Xf 血清は XCf1 に対しては全く不活化作用を示さなかった。XCf2 と Xf との間には血清学的に弱い相互関係が認められたが、XCf1 および XCf2 と Xf2 との間には血清学的類縁関係は全く認められなかった。実験に供した *X. campestris* pv. *citri* 33菌株中、10菌株が XCf1 を、また4菌株が XCf2 を産生した。