Mutation of Histidine Synthesis Induced by Filamentous Phage Cf16 in *Xanthomonas campestris* pv. *citri*

Wen-Chuan Wu and Feng-Hsiang Yang

Graduate Institute of Plant Pathology, National Chung Hsing University, Taichung, Taiwan 402, R.O.C.

ABSTRACT

Wu, W. C., and Yang, F. H. 1994. Mutation of histidine synthesis induced by filamentous phage Cf16 in *Xanthomonas campestris* pv. *citri*. Plant Pathol. Bull. 3:1-8.

Filamentous phage Cf16 induced mutation of histidine synthesis in strain XW45 of *Xanthomonas campestris* pv. *citri* at the frequency of 10^{-1} after lysogenization. All or most of the His⁻ mutants, unlike their wild-type strain XW45, did not utilize citrate, did not decompose the carbohydrates tested, and showed slight decrease in virulence to Liucheng sweet orange; one of them was not tolerant to 3% NaCl. All the His⁻ mutants, when reverted, were still lysogenic for Cf16, and most of the His⁺ revertants did not restore these alterated phenotypic expressions. The wild-type strain XW45 harbored plasmids pXW45N and pXW45J, which fused to become a large plasmid and then dissociated into two plasmids in some of the His⁻ mutants and the His⁺ revertants. The Cf16 prophage was not observed existing as plasmid state in the cells of His⁻ mutants and His⁺ revertants. Thus, the mutation of histidine synthesis in *X. campestris* pv. *citri* XW45 induced by Cf16 is probably due to integration of its prophage at a certain site other than the affected genes on the host chromosome.

Key words: *Xanthomonas campestris* pv. *citri*, filamentous phage Cf16, mutation.

INTRODUCTION

As part of investigations to establish the genetic system of *Xanthomonas campestris* pv. *citri*, Wu (29,30) and Wu and his coworker (34) have reported in Japan that the genetic characteristics of strain XCJ19 of this bacterium, after lysogenized with its phage PXC7, are modified by alteration from smooth to dwarf colony types accompanied with changes in its cell disposition, adsorption to phage PXC7, sensitivity to phage CP2, and virulence to summer orange. In Taiwan, Wu *et al.* (38) have found that filamentous phage Cf16 induces mutation of cysteine synthesis in strain XW47 of this bacterium probably by integration of its prophage into the affected genes on its host chromosome. The mutation also accompany with changes in some biochemical and pathological characteristics, but the mutation and most of the accompanied changes revert with loss of the Cf16 prophage. Later, the phage Cf16 has also been found to induce mutation of histidine synthesis in strain XW45 of this bacterium (35). A study was then initiated to find out whether such His⁻ mutation can revert. The resulting His⁺ revertants were further tested with their His⁻ mutants and wild-type strain XW45 to determine whether they accompany with changes in biochemical, pathological, and molecular-biological characteristics. Part of the results has already been presented in abstract (33,36) and review (32) forms.

MATERIALS AND METHODS

Media and chemical reagents

Nutrient broth, nutrient agar, and agar were the product of Difco Laboratories, Detroit, Michigan, U.S.A. Soft agar, saline, minimal agar, semi-enriched minimal agar (38), and L broth (17) have previously been described. Panvitan, compositive vitamins, was produced from Takeda Yakuhin Co. Adenine, thymine, guanine, cytosine, uracil, casamino acids, L-amino acids, and acridine orange were purchased from Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Bacterial strains

X. campestris pv. *citri* XW45, its His⁻ mutants (35), and their His⁺ revertants obtained in this study were kept at room temperature in darkness in soft agar stab in corked vials, the tops of which were sealed with paraffin (31). All the bacterial strains were removed

from the preserved vials and streaked onto the semi-enriched minimal agar plates, and the grown cultures were kept at 4 C and transfered every 26 weeks. Before use, discrete colonies of each strain were picked up and suspended in saline and streaked onto the minimal agar plates supplemented with or without L-histidine. Unless otherwise stated, all cultures were incubated at 30 C.

Phage

Phage Cf16 was isolated from the lysogenic strain XW16 of *X. campestris* pv. *citri* and propagated on strain XW47 (38). Phage lysate was prepared from semiconfluent lysis on the top-layer nutrient agar plates, filtered through a 0.45 μm Millipore membrane (7), titered by the "drop-on-top-layer" method (30), and then stored at 4 C. Before use, the phage stock was diluted with nutrient broth to appropriate titers.

Lysogenization

Phage Cf16 was added to the cells of strain XW45 suspended in nutrient broth at the concentration of 10^8 cells/ml at the multiplicity of infection (m.o.i.) of 20. Control portion was added with nutrient broth only. After incubation for 24 hr, samples were removed from the mixtures and streaked onto nutrient agar plates. Colonies appeared after 72 hr were streaked three times for single-colony isolation on the nutrient agar plates and then examined for their lysogeny by the method previously described (29).

Curing of Cf16 prophage

The cells of the His$^+$ revertants were suspended in saline at the concentration of 10^3–10^4 cells/ml. Aliquots of 0.2 ml of them were added to 2 ml of nutrient broth containing acridine orange at the concentrations of 25, 50, 75, 100, 150, and 200 μg/ml, respectively. Cultures were incubated with shaking for 48 hr. Control was made in the nutrient broth without adding acridine orange. Appropriate dilutions were spread onto the nutrient agar plate, and the cells from the colonies appeared were examined for their lysogeny.

Identification of nutritional requirements

The Cf16-induced His$^-$ mutants were examined for their nutritional requirements on the minimal agar supplemented first with 5 mg/ml Panvitan, 0.2 ml of the purine and pyrimidine solutions, i.e., 2.5 mg/ml each of adenine, thymine, guanine, cytosine, and uracil (29), or 10 mg/ml casamino acids, secondly with alternate groups of L-amino acids, i.e., 20 μg/ml each of 1) arginine, cysteine, cystine, methionine, 2) isoleucine, leucine, valine, 3) phenylalanine, tryptophan, tyrosine, 4) aspartic acid, glutamic acid, histidine, threonine, 5) alanine, glycine, proline, and serine, and finally with aspartic acid, glutamic acid, histidine, or threonine (20).

Biochemical tests

Colony characteristics were examined on agar plates 72 hr after incubation. Discrete colonies of each of the bacterial strains were suspended in 1 ml of saline at the concentration of approximately 10^8 cells/ml. A loopful suspension of each bacterial strain was used as an inoculum for the tests of gelatin liquefaction, citrate utilization, pectolytic enzyme (12), tolerance to 3% NaCl solution (28), and decomposition of carbohydrates (11).

Inoculation of citrus plants

The cells of the wild-type strain XW45, its Cf16-induced His$^-$ mutants, and their His$^+$ revertants at late log phase were collected by centrifugation at 3,000 rpm. for 30 min., washed twice with saline, resuspended in saline to about 5 $\times$ 10^8 cells/ml, and inoculated by the multiple needle method (30) into young leaves of Liucheng sweet orange (*Citrus sinensis* cv. Liucheng). The inoculated plants maintained in a greenhouse were covered with moist polyethylene bags for two days. The plants were observed at intervals for canker development.

Detection of plasmid and phage DNAs

The alkaline lysis method of Birnboim (1) was employed to isolate plasmid and phage DNAs from the cells of *X. campestris* pv. *citri* XW45, its Cf16-induced His$^-$ mutants, and their His$^+$ revertants grown in L broth at late log phase. The plasmid and phage DNAs were each suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Electrophoresis was carried out with 0.8% agarose gel in a horizontal tank at 95 volts for 3–4 hr. The gel was then stained in 0.5 μg/ml solution of ethidium bromide for 30 min. The DNA bands were photographed under an UV light at the wave length of 254 nm. Under the same conditions, *Escherichia coli* with plasmid RP4 (3.4 $\times$ 10^7 daltons), obtained from Dr. K. J. Li, Department of Soil Science, National Chung Hsing University, and *Salmonella typhimurium* trpA512 with plasmids F'lac$^+$ and pSTL512 (7.4 and 6.0 $\times$ 10^7 daltons, respectively) grown at 37 C were used as standards.

RESULTS

Mutation of histidine synthesis in *X. campestris* pv. *citri* XW45 after lysogenized with phage Cf16

Strain XW45 of *X. campestris* pv. *citri*, after streaked onto the minimal agar plates, produced circular, convex, entire, glistening, smooth, yellow colonies. When cells of the smooth colonies of strain XW45 were suspended in nutrient broth at the concentration of 10^8 cells/ml and mixed with phage

Cf16 at the m.o.i. of 20, incubated for 24 hr, and streaked onto the nutrient agar, all the colonies appeared were smooth yellow and become lysogenic for phage Cf16. One hundred lysogenic smooth colonies were picked up at random and streaked onto the semi-enriched minimal agar plates, on which 13 white

TABLE 1. Colony characteristics of Cf16 phage-induced His⁻ mutants and their wild-type strain XW45 in *Xanthomonas campestris* pv. *citri* on nutrient agar, minimal agar supplemented without or with various nutrients, and semi-enriched minimal agar plates

Medium and Nutrients supplemented	Colony characteristics[1]	
	Wild-type strain XW45	His⁻ mutants XW45(CF16)-4-16
Nutrient agar	SY	SY
Minimal agar (MA)	SY	—
Semi-enriched MA	SY	WM
MA+(Panvitan)[2] or + (Adenine, thymine, guanine, cytosine, or uracil)[3]	SY	—
MA+(Casamino acids)[4]	SY	SY
MA+(L-aspartic acids, L-glutamic acid, L-histidine, or L-threonine)[5]	SY	SY
MA+L-histidine[5]	SY	SY

[1] SY and WM, smooth yellow and white minute colonies produced, respectively. —, no growth.
[2] Supplemented at concentration of 5 mg/ml.
[3] Supplemented at concentration of 2.5 mg/ml.
[4] Supplemented at concentration of 10 mg/ml.
[5] Supplemented at concentration of 20 µg/ml.

TABLE 2. Frequencies of His⁺ reversion from the Cf16-induced *Xanthomonas campestris* pv. *citri* His⁻ mutants after preserved in soft-agar stabs for one year and their lysogeny for phage Cf16

His⁻ mutants	Frequencies of reversion (x 10⁻¹)	Lysogeny for phage Cf16[1]
XW45(Cf16)-11	6.5	+
-12	5.1	+
-13	2.2	+
-14	6.1	+
-16	8.2	+

[1] +, positive.

minute colonies were obtained and designated with the prefix XW45 (Cf16) followed by the number, and, after examined on the minimal agar for their nutritional requirements, L-histidine was found to be functional for them to produce smooth yellow colonies (Table 1). The mutants XW45 (Cf16)-4 to 16 are deficient in histidine synthesis and, hence, they were genetically designated His⁻ mutants. They were induced by phage Cf16 at the frequency of 0.13.

Reversion of the histidine synthesis mutation

Of the 13 His⁻ mutants, after preserved at room temperature in darkness in soft-agar stabs for one year, His⁻ mutants XW45 (Cf16)-11, 12, 13, 14, and 16 were examined and found to revert to produce His⁺ colonies on the semi-enriched minimal agar at the frequencies of 6.5, 5.1, 2.2, 6.1, and 8.2 × 10⁻¹, respectively. His⁺ revertants thus obtained were designated as XW45-11R, 12R, 13R, 14R, and 16R, respectively, followed by the number. All the His⁺ revertants were still lysogenic for phage Cf16, i.e., not cured of the Cf16 prophage (Table 2). They were not cured of the Cf16 prophage even after treated with acridine orange at the concentrations of 25, 50, 75, 100, 150, and 200 µg/ml, respectively, or preserved again in the soft-agar stabs for one year.

Biochemical characteristics of the Cf16-induced His⁻ mutants and their His⁺ revertants

The His⁻ mutants and their His⁺ revertants were examined with their wild-type strain XW45 for biochemical reactions (Table 3). All or most of these His⁻ mutants, unlike their wild-type strain XW45, changed their reactions from positive to negative in citrate utilization and decomposition of trehalose, xylose, mannitol, mannose, glycerol, fructose, and lactose. His⁻ mutant XW45 (Cf16)-11 was not tolerant to 3% NaCl, and His⁻ mutants XW45 (Cf16)-14 and 16 decomposed trehalose, xylose, and mannitol. All or most of the His⁺ revertants, like their His⁻ mutants but unlike their wild-type strain XW45, reacted negatively in utilization of citrate and decomposition of the carbohydrates tested and positively in tolerance to 3% NaCl.

Virulence of the Cf16-induced His⁻ mutants and their His⁺ revertants

The Cf16-induced His⁻ mutants of *X. campestris* pv. *citri* XW45 and their His⁺ revertants were inoculated into the leaves of Liucheng sweet orange to compare their virulence with wild-type strain XW45 (Table 4). Wild-type strain XW45 as well as most of its His⁻ mutants and their His⁺ revertants developed canker lesions 3 days after inoculation, and the number of lesions increased rapidly and reached the maxima, 100% of infection, within 7 or 14 days. His⁻ mutant XW45 (Cf16)-14 and His⁺ revertants XW45-11R-3 and 12R-12, however, did not show canker lesions 3 or 7

TABLE 3. Biochemical characteristics of the Cf16-induced His⁻ mutants, their His⁺ revertants, and wild-type strain XW45 in *Xanthomonas campestris* pv. *citri*

Biochemical characteristics[1]	Wild-type strain XW45	His⁻ mutants	His⁺ revertants
Citrate utilization	+	−	−
Tolerance to 3% NaCl	+	+,−[2]	+
Decomposition of carbohydrates			
Galactose	+	+	+,−[2]
Trehalose	+	−,+[2]	−
Xylose	+	−,+[2]	−
Mannitol	+	−,+[2]	−
Mannose	+	−	−
Glycerol	+	−	−
Fructose	+	−	−
Lactose	+	−	−

[1] +, positive reaction; −, negative reaction.

[2] The His⁻ mutant XW45 (Cf16)-11 was not tolerant to 3% NaCl; the His⁺ revertant XW45-13R-18 did not decompose galactose; the His⁻ mutants XW45 (Cf16)-14 and 16 decomposed trehalose, xylose, and mannitol.

days after inoculation, but developed lesions and reached 100% of infection within 14 days. The lesions developed by most of the His⁻ mutants and the His⁺ revertants were smaller than those by the wild-type strain XW45. Comparison of canker development and size of the canker lesion by these bacterial strains after inoculation showed slight decrease in the virulence of most of the His⁻ mutants and still did not revert to normal in the His⁺ revertants.

Plasmids and Cf16 prophage in the Cf16-induced His⁻ mutants and their His⁺ revertants

Plasmid DNAs existed in *X. campestris* pv. *citri* XW45, its Cf16-induced His⁻ mutants, and their His⁺ revertants, with *E. coli* and *S. typhimurium* as standards, are shown in Fig. 1. The cells of *X. campestris* pv. *citri* XW45, its Cf16-induced His⁻ mutants XW45 (Cf16)-11, 13, and 16 (pXW45N band of the latter was seen faintly), and His⁺ revertants XW45-11R-1, 13R-18, and 14R-20 were found to harbor plasmids pXW45N and pXW45J with molecular weight of 3.5 and 2.5 × 10⁷ daltons, respectively. Those of His⁻ mutant XW45 (Cf16)-12 harbored an additional plasmid, namely pXCl, with molecular weight of 5.8 × 10⁷ daltons, but lost plasmid pXW45N when reverted to normal. Those of

His⁻ mutant XW45 (Cf16)-14 as well as His⁺ revertants XW45-12R-9 and 16R-25 (pXW45J band of the latter was seen faintly) harbored plasmids pXCl and pXC45J, and XW45 (Cf16)-14 lost plasmid pXCl but regained plasmid pXW45N when reverted. Cf16 prophage was not observed as plasmid state in the cells of His⁻ mutants and His⁺ revertants.

DISCUSSION

Phage-induced mutations have been studied extensively in *E. coli* K-12. Phages λ (22,24,25), Mu (2), and D108 (9), after lysogenization, cause genetic modification in their host by insertion of their prophages within certain or uncertain sites of the affected structural genes, thus destroying the continuity of the genetic information. The Mu-induced mutation mediates the formation of chromosomal rearrangements such as deletion. inversion, duplication, and transposition of host DNA segments, as well as replicon fusion (26). The λ-induced mutants can revert with curing of λ prophage (21,22); whereas, the Mu-induced mutants revert but the prophage become defective (15,21). Phage-induced mutations have also been reported in *Vibrio cholerae* (14) and *Agrobacterium tumefaciens* (8). A comparable phenomenon has also been observed in *X. campestris* pv. *citri*. After lysogenized with filamentous phage Cf16, strain XW47 of this bacterium undergoes mutation of cysteine synthesis, which have accompanied with altered phenotypic expressions in citrate utilization, tolerance to 3% NaCl, and galactose, trehalose, mannitol, xylose, mannose, glycerol, dextrin, and raffinose decomposition, as well as decrease in virulence to Liucheng sweet orange. The Cys⁻ mutants can revert with loss of lysogeny, and most of the altered phenotypic expressions are restored (38). Cf16 prophage has been evidenced to integrate into the chromosome of *X. campestris* pv. *citri* (5). All of these results suggest that such a mutation induced by phage Cf16 is probably due to integration of its prophage at the affected *cys* genes (38), and integration of the Cf16 prophage, as shown in some of transposons and insertion sequences (6,10,19,39), may act as switch for turning off or on of the genes.

Mutation of histidine synthesis induced by phage Cf16 in strain XW45 of *X. campestris* pv. *citri* also loses ability of citrate utilization; some of the His⁻ mutants do not decompose the carbohydrates tested, and some show change in tolerance to 3% NaCl, as well as decrease in virulence to Liucheng sweet orange. The His⁻ mutation, however, reverts without loss of lysogeny for phage Cf16, and most of the His⁺ revertants have not restored the altered phenotypic expressions in the biochemical and pathological characteristics. Acridine orange (13) or soft-agar preservation (30,38), although having cured some

TABLE 4. Canker development and lesion size on Liucheng sweet orange leaves inoculated with *Xanthomonas campestris* pv. *citri* XW45, its Cf16-induced His⁻ mutants, and their His⁺ revertants

Bacterial strain	% Infection (days) after inoculation			Diameter of canker lesion (mm) 21 days after inoculation
	3	7	14	
Wild-type strain XW45	100	100	100	2.4 a
His⁻ mutants				
XW45(Cf16)-11	66.3	98.8	100	2.0 b
-12	98.0	100	100	2.3 a
-13	92.2	100	100	2.1 b
-14	0	27.5	100	2.0 b
-16	94.4	96.3	100	1.8 b
His⁺ revertants				
XW45-11R-1	95.0	95.0	100	2.2 a
-2	97.5	100	100	1.9 b
-3	0	0	100	1.3 b
XW45-12R-7	100	100	100	1.9 b
-11	98.8	100	100	1.9 b
-12	0	0	100	1.7 b
XW45-13R-13	100	100	100	1.7 b
-14	100	100	100	2.1 b
-15	98.8	100	100	1.9 b
XW45-14R-19	97.5	100	100	1.8 b
-20	98.8	100	100	2.2 a
-21	100	100	100	2.1 b
XW45-16R-25	90.0	93.8	100	1.9 b
-26	81.3	82.5	100	1.9 b
-27	92.5	98.8	100	2.0 b

[1] Each bacterial strain was inoculated into 5 leaves, each wounded with 20 needles set on a rubber stopper in the distance of 5×5 mm.

[2] Figures followed by different letters denote a significant difference at 5% level from that of the wild-type strain XW45 according to the Student's *t* test.
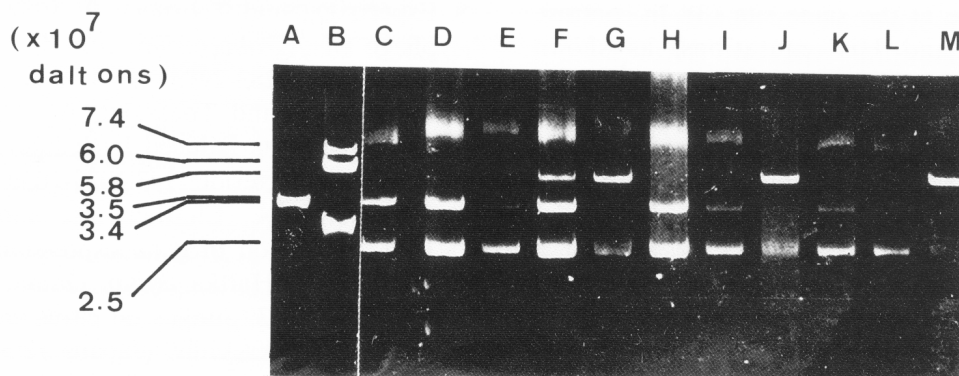

Fig. 1. Agarose gel electrophoresis of plasmid DNAs isolated from (A) *Escherichia coli*/RP4 ($3.4×10^7$ daltons), (B) *Salmonella typhimurium trpA512*/F′*lac*′ and pSTL512 (7.4 and $6.0×10^7$ daltons, respectively), and (C) *Xanthomonas campestris* pv. *citri* XW45, (D) its Cf16-induced His⁻ mutants XW45 (Cf16)-11, (F) 12, (H) 13, (J) 14, and (L) 16, and (E) their His⁻ revertants XW45-11R-1, (G) 12R-9, (I) 13R-18, (K) 14R-20, and (M) 16R-25.

plasmids or prophages of some phages, have failed to cure Cf16 prophage in the His$^+$ revertants. Cf16 prophage has also not observed as plasmid state in its host cells. All of these results, therefore, suggest that the His$^-$ mutation induced by phage Cf16 may be due to the integration of its prophage into a certain site other than the *his* genes on the host chromosome. Furthemore, wild-type strain XW45, its Cf16-induced His$^-$ mutants, and most of their His$^+$ revertants harbor two plasmids, which have previously been designated as pXW45N and pXW45J (27); whereas, one of the His$^-$ mutants harbors an additional plasmid, as designated here as pXCl, but it loses plasmid pXW45J when reverts to normal. One of the His$^-$ mutants and two of the His$^+$ revertants harbor plasmids pXCl and pXW45J, and the former loses plasmid pXCl but regains plasmid pXW45N when reverts. Size of plasmid pXCl was approximately the sum of plasmids pXW45N and pXW45J, revealing that, as shown by Novick (18) and Wu *et al.* (37), two heterogenic plasmids might fuse and then dissociate in their host cells. Plasmids pXW45N and pXW45J carry transposable elements ISX$C4$ and ISX$C5$, respectively (27). Fusion and dissociation of these two plasmids in the host cells are probably due to these transposable elements. Since the molecular weight of the RF DNA of Cf16 is 4.8 × 10^6 daltons (16), and those of plasmids pXCl, pXW45N, and pXW45J are 5.8, 3.5, and 2.5 × 10^7 daltons, respectively, the latters are not relate to the Cf16 prophage. The Cf16 prophage, therefore, is not existing as plasmid state in its host cells. It may, therefore, exist as integrated state into the host chromosome.

Mutagenesis by Cf16 is similar in several respects with that induced by λ phage, but different from that induced by phage Mu. First, Mu displays no strong affinity for one gene over another (25); moreover, even within a single gene, Mu has never been observed to insert more than once at the same site (3). In contrast, Cf16 induces Cys$^-$ and His$^-$ mutations, as shown previously (38) and in this study, at more frequently than any others. Second, Cf16-induced Cys$^-$ and His$^-$ mutations, unlike those induced by Mu, but like those by λ, are reversible. Mutations induced by insertion of the prophage λ is demonstrated in *E. coli* deleted for the λ attachment site (23). Dai *et al.* (4) has shown that Cf16 prophage has specific insertion site at the chromosome of *X. campestris* pv. *citri* XW47. Nevertheless, the mechanism of Cf16 induced-Cys$^-$ and His$^-$ mutations in the *X. campestris* pv. *citri* cells accompanied with the altered phenotypic characteristics needs further studies.

ACKNOWLEDGEMENTS

LITERATURE CITED

1. Birnboim, H. C. 1983. A rapid alkaline extraction method for the isolation of plasmid DNA. Pages 243-255 in: Methods in Enzymology, Vol. 100, R. Wu, L. Grossman, and K. Moldave, eds. Academic Press, Inc. New York, U.S.A.

2. Boram, W., and Abelson, J. 1971. Bacteriophage Mu integration: on the mechanism of Mu-induced mutations. J. Mol. Biol. 62:171-178.

3. Bukhari, A. I., and Zipser, D. 1972. Random insertion of Mul DNA within a single gene. Nature (London) New Biol. 236:240-243.

4. Dai, H., Chow, T. Y., Liao, H. J., Chen, Z. Y., and Chiang, K. S. 1988. Nucleotide sequences involved in the neolysogenic insertion of filamentous phage Cf16-vl into the *Xanthomonas campestris* pv. *citri* chromosome. Virology 167:613-620.

5. Dai, H., Tsay, S. H., Kuo, T. T., Lin, Y. H., and Wu, W. C. 1987. Neolysogenization of *Xanthomonas campestris* pv. *citri* infected with filamentous phage Cf16. Virology 156:313-320.

6. Davis, B. D., and Dulbecco, R. 1981. Bacterial and molecular genetics. Pages 129-285 in: Microbiology, 3rd ed., B. D. Davis, R. Dulbecco, H. N. Eisen, and H. S. Ginsberg, eds. Harper and Row, Publicashers, Inc. Hagerston, U.S.A.

7. Eisenstark, A. 1967. Bacteriophage techniques. Pages 449-524 in: Methods in Virology, Vol. I, K. Maramorosch, and H. Koprowski, eds. Academic Press, Inc., New York, U.S.A.

8. Expert, D., and Tourneur, J. 1982. ϕ , a temperate phage of *Agrobacterium tumefaciens*, is mutagenic. J. Virol. 42:283-291.

9. Faelen, M., and Toussaint, A. 1980. Chromosomal rearrangements induced by temperate bacteriophage D108. J. Bacteriol. 143:1029-1030.

10. Glansdorff, N., Charlier, D., and Zafarullah, M. 1981. Activation of gene expression by IS2 and IS3. Cold Spring Harbor Symp. Quant. Biol. 45:153-156.

11. Goto, M. 1962. Studies on citrus canker I. Bull. Fac. Agric. Shizuoka Univ. (Japan) 12:3-72. (In Japanese with English summary)

12. Harrigan, W. F., and McCance, M. E. 1966. Biochemical tests for bacteria. Pages 51-68 in: Laboratory Methods in Microbiology. Academic Press, Inc., New York, U.S.A.

13. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. 46:57-64.

14. Johnson, S. R., Liu, B. C. S., and Romig, W. R. 1981. Auxotrophic mutations induced by *Vibrio cholerae* mutator phage VcA1. FEMS Microbiol. Lett. 11:13-16.

15. Khatoon, H., and Bukhari, A. I. 1981. DNA rearrangements associated with reversion of bacteriophage Mu-induced mutations. Genetics 98:1-24.

16. Lin, Y. H. 1983. Studies on the Integration of the Filamentous Phage Cf Genome into the Host Chromosome. M.S. Thesis, National Taiwan University, Taipei, Taiwan, R.O.C. (in Chinese)

17. Miller, J. H. 1972. Experiments in Molecular Genetics, p. 431-435. Cold Spring Harbor. New York, U.S.A.

18. Novick, R. P. 1969. Extrachromosomal inheritance in bacteria. Bacteriol. Rev. 33:210-263.

19. Saedler, H., Reif, H. J., Hu, S., and Davidson, N. 1974. IS2, a genetic element for turn-off and turn-on of gene activity in *E. coli*. Mol. Gen. Genet. 132:265-289.

20. Seaman, G. R. 1963. Experiments in Microbial Physiology and Biochemistry, p. 10-24. Burgess Publishing Co. Minneapolis, U.S.A.

21. Shimada, K. 1975. *E. coli* mutants produced by the insertion of bacteriophage DNA. Protein, Nucleic Acid, and Enzyme (Tokyo) 20:683-690. (in Japanese)

22. Shimada, K., and Weisberg, R. A. 1972. Prophage lambda at unusual chromosomal locations I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. 63:483-503.

23. Shimada, K., and Weisberg, R. A. 1973. *E. coli* mutants produced by the insertion of bacteriophage lambda DNA. Genetics 73 (Suppl.): 81-83.

24. Shimada, K., Weisberg, R. A., and Gottesman, M. E. 1973. Prophage lambda at unusual chromosomal locations II. Mutations induced by bacteriophage lambda in *E. coli* K12. J. Mol. Biol. 80:297-314.

25. Taylor, A. L. 1963. Bacteriophage-induced mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 50:1043-1051.

26. Toussaint, A., and Resibois, A. 1983. Phage Mu: transposition as a life-style. Pages 105-158 in: Mobile Genetic Elements, J. A. Shapiro, ed. Academic Press, Inc. New York, U.S.A.

27. Tu, J., Wang, H. R., Chang, S. F., Charng, Y. C., Lurz, R., Dobrinski, B., and Wu, W. C. 1989. Transposable elements of *Xanthomonas campestris* pv. *citri* originating from indigenous plasmids. Mol. Gen. Genet. 217:505-510.

28. Tzeng, K. C., and Hsu, S. T. 1981. Identification and characterization of soft-rotting *Erwinia* in Taiwan. Plant Prot. Bull. (Taiwan, R.O.C.) 23:77-85.

29. Wu, W. C. 1972. Phage-induced alteration of colony type in *Xanthomonas citri*. Ann. Phytopathol. Soc. Jpn. 38:146-155.

30. Wu, W. C. 1972. Phage-induced alterations of cell disposition, phage adsorption and sensitivity, and virulence in *Xanthomonas citri*. Ann. Phytopathol. Soc. Jpn. 38:333-341.

31. Wu, W. C. 1975. Bacterial genetics: introduction. Bull. Soc. Plant Pathol. Nat. Chung Hsing Univ. (Taiwan, R.O.C.) 6:95-101. (in Chinese)

32. Wu, W. C. 1988. Citrus canker in Taiwan. Pages 15-25 in: Proceedings of International Symposium of Citrus Canker, Decline/Blight and Similar Diseases, V. Rosetti coordinated. Fundacao Cargill. Campinas, Sao Paulo, Brazil.

33. Wu, W. C. 1988. Mutation induced by filamentous temperate phage Cf16 in *Xanthomonas campestris* pv. *citri*. Genome 30 (Suppl. 1):423. (Abstr.)

34. Wu, W. C., and Iino, T. 1972. Phage-induced alterations in *Xanthomonas citri*. Ann. Rep. Nat. Inst. Genet. (Japan) 22:18-19. (Abstr.)

35. Wu, W. C., and Tan, Y. Y. 1987. Phage-induced mutations of histidine and cysteine syntheses in *Xanthomonas campestris* pv. *citri*. Pages 584-593 in: Plant Pathogenic Bacteria, E. L. Civerolo, A. Collmer, R. E. Davis, and A. G. Gillaspie, eds. Martinus Nijhoff Publishers. Dordrecht, Netherlands.

36. Wu, W. C., and Yang, F. H. 1987. His⁻ mutantion in *Xanthomonas campestris* pv. *citri* induced by phage Cf16. Chin. J. Microbiol. Immunol. 20:M-22. (Abstr.) (in Chinese)

37. Wu, W. C., Hung, M. N., and Wang, L. Y. 1990. Characterization of the phage-induced *Xanthomonas campestris* pv. *citri* mutants. Plant Prot. Bull. (Taiwan, R.O.C.) 32:289-297. (in Chinese).

38. Wu, W. C., Hong, Y. R., Huang, C. M., Tan, Y. Y., Lee, C. I., Chang, S. F., Wang, C. H., and Huang, M. L. 1985. Phage-induced mutations in *Xanthomonas campestris* pv. *citri*. Plant Prot. Bull. 27:19-33.

39. Zafarullah, M., Charlier, D., and Glansdorff, N. 1981. Insertion of IS3 can "turn-on" a silent gene in *Escherichia coli*. J. Bacteriol. 146:415-417.

摘　　要

吳文川、楊鳳香．1994．柑桔潰瘍病菌由Cf16絲狀噬菌體誘導的組氨酸合成突變．植病會刊3: 1-8．（台中市　國立中興大學植物病理學研究所）

　　柑桔潰瘍病菌 (*Xanthomonas campestris* pv. *citri*) 的 XW45 菌株，被 Cf16 絲狀噬菌體潛溶化後，以約爲 10^{-1} 的頻率產生 His⁻ 突變菌株。全部或部分 His⁻ 突變菌株，與 XW45 野生型菌株不同，不能利用檸檬酸鹽，不能分解海藻糖、木糖、甘露醇、甘露糖、甘油、果糖和乳糖，且其對柳橙的致病力稍微降低，而部份 His⁻ 突變菌株仍然不能在 3% 氯化鈉的培養基上生長。當此等 His⁻ 突變菌株回復時，未失去其潛溶性，而大部分 His⁺ 回復菌株依然保持此等變化的特性。XW45 野生型菌株所含有 pXW45N 和 pXW45J 二個質體，在有些 His⁻ 突變菌株及 His⁺ 回復菌株細胞內，能融合成爲另一質體或再分裂成爲原來的質體。Cf16 噬菌體在 His⁻ 突變菌株和 His⁺ 回復菌株細胞內均不呈顯游離質體狀態的 DNA；因此，可推測其原噬菌體可能插入寄主染色體受影響基因以外的一定位置上。

關鍵詞： 柑桔潰瘍病菌、Cf16 絲狀噬菌體、突變。