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PROPERTY OF Cf16 LYSOGEN[1,2]

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Abstract

The length of Cf16 appears to be 1314±203 nm by measuring 105 phage particles under the electron microscope. Following Cf 16 infecion into host cell, ***Xanthomonas campestris*** pv. ***citri***, the neolysogenization process was carried out. Cf16 genome may integrate into host chromosome and stay as a temperate phage.

The Cf16-lysogen, L10, produced phage titer, about 2×10^6 PFU/ml, in stationary phase cell culture. UV, heat and mytomycin C were not able to induce Cf16 from the L10 lysogen. Acridine orange and anti-phage serum treatments could not cure the phage from L10 lysogen.

Key words: Filamentous phage Cf16, Cf16-lysogen, *Xanthomonas campestris* pv. *citri*.

Filamentous phage Cf 16 was first isolated from *Xanthomonas campestris* pv. *citri* XW 16 by Wu *et al.* (1985). The length of Cf 16 is 1314±203 nm by measuring 105 phage particles under the electron microscope (Fig. 1). It appears to be about 300 nm longer than another filamentous phage Cf isolated from the same host (Dai *et al.*, 1980). After the infection of Cf 16 into indicator strain, *Xanthomonas campestris* pv. *citri* XW 47, the cells were plated and the phage carrier colonies were picked and followed by several successive single colony transfers. For each transfer, the liquid culture was made and the phage titer was tested. It was found that some of the tested culture showed phage titer about 1×10^5 to 5×10^6 PFU/ml. It was about 10^{-3} to 10^{-4} of phage carrier cell and resembled a lysogenic system. Southern hybridization analysis showed that the phage Cf 16 genome was integrated into the host chromosome (Dai *et al.*, 1986). One lysogen, L10, produces low phage titre, about 2×10^6 PFU/ml in LB medium (10 g tryptone, 5 g yeast extract, 5 g NaCl and 1 g glucose in 1 liter water) is characterized and reported here.

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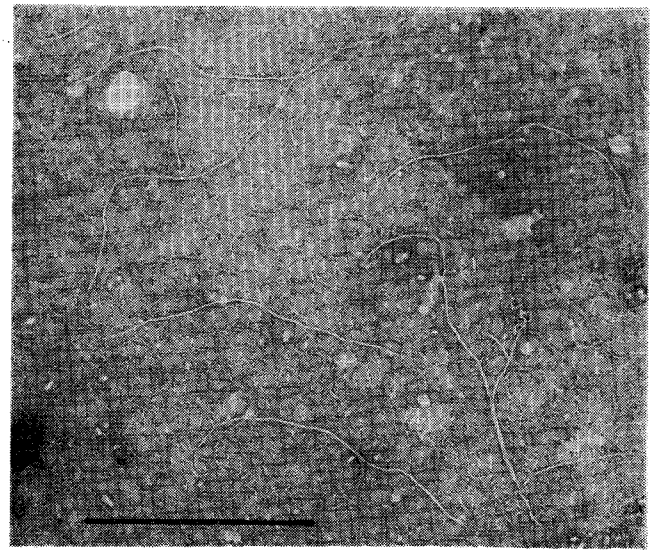

Fig. 1. The phage suspension was mixed with equal volumn of 0.1 M ammonium acetate. A drop of the mixture was applied on a grid coated with Formvar and carbon film. The excessive solution was sponged off with filter paper. The specimen was negative stained with 2% phosphotungstic acid and then examined under a Hitchi H-600 electron microscope. With a sample size of 105, the length of Cf16 is measured to be 1314±203 nm (Bar represents 1 micron.)

The logarithmic phase cells of L10 were well washed or treated with anti-phage serum to get rid of the external free phages. The cells were resuspended into fresh LB medium and incubated at 28°C with shaking. The infective center and colony forming number were determined along the incubation time. Figure 2 shows that one infective center formed every 10^3 to 10^4 cells in spite of all the cells carrying the phage-producing capacity. 100 colonies were tested for each testing point and all of them were able to produce phage and immune to further Cf16 infection. This means only one cell out of 10^3 to 10^4 phage-carrier cells capable to develop the active phage. If Cf16 is propagated and released from free RF (replicative form) like the other filamentous phages, the number of infective center should be equal to the number of carrier cells (Hsu, 1967). It is not the case. We believe that Cf16 is released from integrated genome resembled to the life cycle of temperate phage.

Various concentrations of mytomcin C were tested for its induction of phage on L10. Only the result of 2 μg/ml mytomycin C treatment is represented here. The cell stage and treatment condition were the same as described in Fig. 2 except mytomycin C was applied along cell incubation. The high concentration of cells was used in order to have more significant phage production along treatment. Figure 3 reveals that about 10 times of colony forming cells register as infective centers after

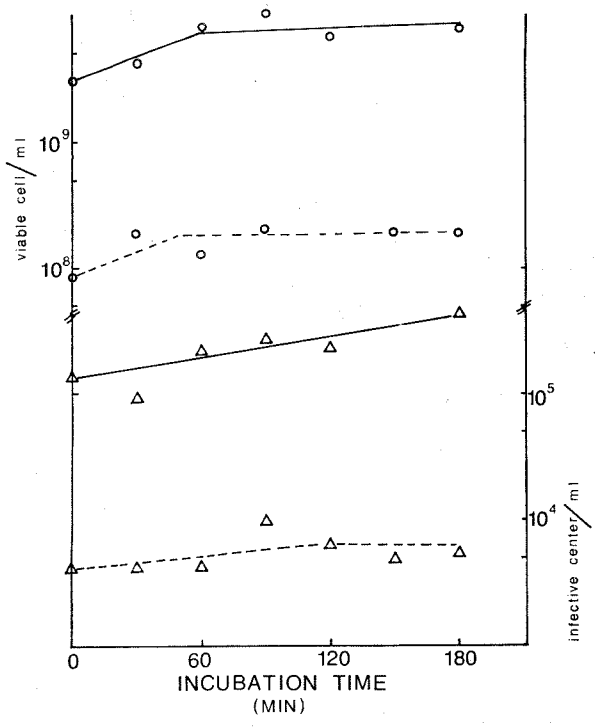

Fig. 2. The rate of L 10 cell developing into active phage-producing cell. Log phase cells were washed twice or treated with anti-phage serum to get rid of external phage particles. The cells were resuspended into fresh LB liquid medium at the concentration of 8×10^7 cells/ml or 3×10^9 cells/ml and iucubated at 28°C with shaking. Along the incubation, the viable cell number and the infective center were determined. The infective center was obtained either by treating the cell with anti-phage serum or by wasing the cell with H_2O. Only the data got from serum treatment are shown in this figure. Five independent experiments have done but only two sets of data are represented here. 100 viable colonies were examined for each testing. All of them were phage carrier and immune to further phage infection as well. Viable colonies: ○---○, start at concentration 8×10^7/ml. ○—○, start at cell concentration 3×10^9/ml. Infective center: △---△, start at cell concentration 8×10^7/ml. △—△, start at cell concentration 3×10^9/ml.

mytomycin C treatment. More phage particles were released per infective center under the same treatment. The exact number of phage production per infective center is unknown since readsorption seems to occur along the incubation (data not shown). 100 mytomycin C-treated colonies and 50 control colonies were tested. All of them showed phage producing capacity and immunity of Cf16. It is difficult for us to conclude that L10 lysogen can be induced by mytomycin C. There may be some cells which fail to form colony but able to form plague on the plate. Our unpublished data showed that UV and heat are unable to cause phage induction as well.

If Cf16 free genome is co-existed with integrated form and if the free form is responded for phage releasing as the production of the other filamentous phages, we should be able to eliminate those plasmids by growing L10 in a medium con-

Table 1. *The treatment of acridine orange on L10*

The log phase cells were incubated with acridine orange and anti-phage serum (K value $=5\times10^3$/min) for 12 h. No free phage could be detected in the medium. The cells with and without washing were plated on the LB medium. Colonies were picked after 3-day incubation and tested for their phage production.

Dose of AO (μg/ml)	Colonies tested	Production of Cf16
2.5	40	all+
	40*	all+
5	30	all+
	40*	all+
10	30	all+
20	30	all+
30	60	all+
	30*	all+
60	150	all+

* Cells without washing before plating.

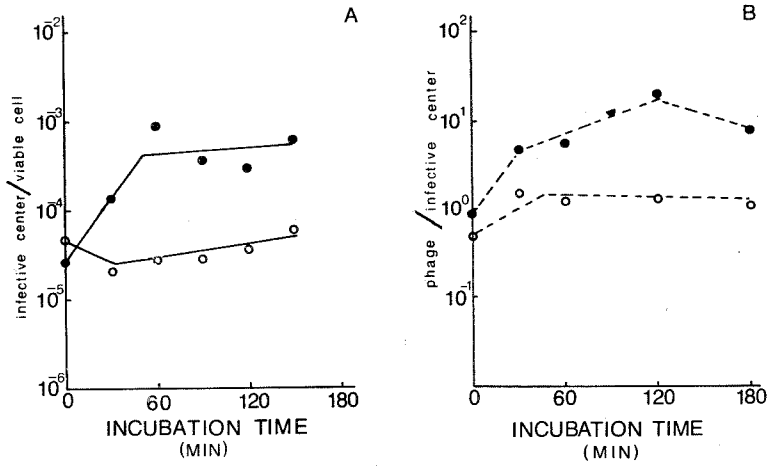

Fig. 3. Phage production and infective center formation per viable cell after mytomycin C treatment. Log phase cells of L10 were washed and concentrated to 3×10^9 cells/ml as described in Fig. 2. 2μg/ml mytomycin C was applied during incubation. The phage titer, infective center, viable colony were determined along incubation. Four independent experiments were carried out. Only one result is shown here. Infective center/viable cell: ●—●, with mytomycin C treatment, ○—○, without mytomycin C tratment, Phage/viable cell: ●---●, with mytomycin C treatmen, ○---○ without mytompcin C treatment.

taining acridine orange (Hsu, 1967). In Table 1, L10 was incubated with series concentrations of acridine orange plus anti-phage serum for 12 h. The cells were plated with and without washing. None of the colony tested showed phage curing character. This result indicates that Cf 16 is produced from a plasmid-like genome co-existed in L10.

The anti-phage serum with a concentration of complete neutralization on Cf16 was also incubation with the L10. After incubation for 12 cell generations, the cells were plated and then tested. All the 100 colonies tested were able to produce phage and immune to further infection (data not shown). This result reveals that phage genome can not be eliminated through cell division in the absence of extracellular phage reinfection.

Recycling of Cf16 lysogen was also investigated. Cf16 purified from L10 were grown on XW 47 (moi=50) in the LB medium. After 2 h incubation at 28°C, the cells were plated. The bacterial colonies carried phage were purified by 8 sequential single colony isolation. The study of phage production, infective center and immunity on those new isolates showed that all the 30 tested isolates had the same character as L10. It is suggested that after Cf16 infection, the phage genome

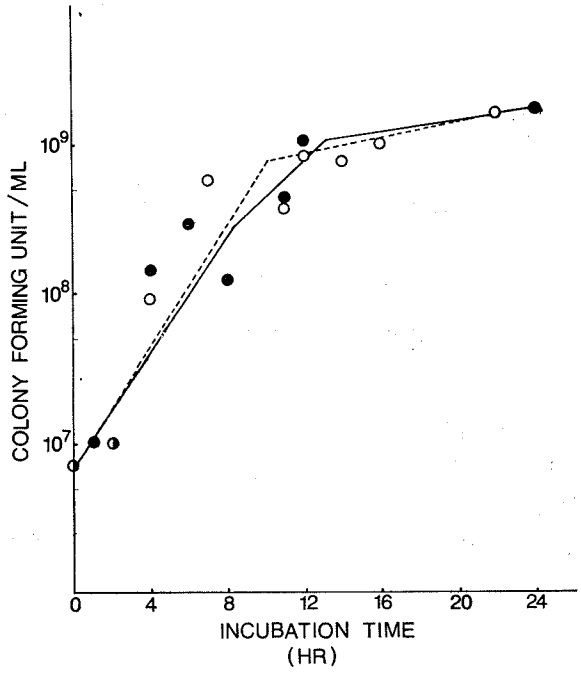

Fig. 4. Growth curves of L10 and XW47. Stationary phase cells were diluted into liquid LB medium at the cell concentration 7×10^6 cells/ml and incubated at 28°C with shaking. Aliquot of cells were taken and diluted properly for colony plating along incubation. ●—●, L10, ○—○, XW47.

may propogated as a free RF in the host cell and at certain stage, the phage integration occurs.

Cf16 propagates in the host without lysing the cell. The growth curves of L10 showed that Cf16-lysogen has the same growth rate as phage free host (Fig. 4).

Unlike other filamentous phages, Cf16 genome may integrate into the host chromosome. About one out of 10^3 to 10^4 lysogenized host may convert to active form and liberate phage. UV, mytomycin C and heat seem unable to achieve phage induction. Anti-phage serum treatment and acridine orange treatment can not cure the phage from lysogen.

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Cf16 潛 溶 菌 的 性 質

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線狀噬菌體 Cf16 在電子顯微鏡下的長度爲 1314±203 nm. 侵入寄主柑桔潰瘍病病原菌後，它可經由「新潛溶作用」將其「基因組」插入寄主染色體而以潛溶性噬菌體方式存在寄主體內。

液態培養之靜止期潛溶菌其產生溶菌斑的能力爲 2×10^6 PFU/ml. 紫外線，加溫和 mytomycin C 都無法由潛溶菌中誘發 Cf16 之釋放。Acridine orange 和抗噬菌血清亦無法除去寄主體內之 Cf16。