日植病報 61：127-129 (1995)
Ann. Phytopathol. Soc. Jpn. 61：127-129 (1995)

Integration of Filamentous Phage Xf2 DNA into Chromosomal DNA of *Xanthomonas campestris* pv. *oryzae*

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Key words: integration, filamentous phage Xf2 DNA, chromosomal DNA, *Xanthomonas campestris* pv. *oryzae*.

A filamentous phage Xf2 was released from many strains of *Xanthomonas campestris* pv. *oryzae*[2]. The concentration of Xf2 in the culture medium of strains N5845 and N5875 was about 1×10^1 and 1.2×10^5 pfu/ml, respectively. An indicator strain N5850, which was artificially infected with Xf2, released phage into the medium at a concentration of about 1×10^{11} pfu/ml[3]. The N5845 and N5875 strains were found to be immune to Xf2 reinfection and the phage productivity of the two strains was stable. This productivity has been maintained for many years of preservation by means of serial subcultures. It has been shown that the filamentous phage Cf16 of *X. c.* pv. *citri* enters a prophage state after serial transfers of infected host bacteria. The phage titer of the lysogen culture was lower than that of the initial culture[1]. The lysogenic phenomenon of the filamentous phage Cflt of *X. c.* pv. *citri* also has been reported[5,6]. From the above-mentioned characteristics of *X. c.* pv. *oryzae* N5845 and N5875, it was suspected that the two strains might be made lysogenic for Xf2 phage, and in the present study Southern hybridization was undertaken to discover if Xf2 DNA would integrate into the host DNA.

The double-stranded replicative form (RF) DNA of Xf2 was prepared from Xf2-infected bacteria by cesium chloride-ethidium bromide density gradient centrifugation as described in a previous study[4]. The RF-DNA which was used as a probe in Southern hybridization was further purified by subjecting it to 0.7% agarose gel electrophoresis and eluting it from the gel with powdered glass. The isolation of total DNA from bacterial cells was carried out as follows: *X. c.* pv. *oryzae* N5845, N5875, N5850 and N5850 infected with Xf2 at a multiplicity of 10 were grown in 20 ml of PS medium[2] at 30°C. The bacteria was collected by centrifugation at 5,000×*g* for 10 min and resuspended in 7 ml of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). Eight-tenths of a milliliter of 5% SDS and 130 μl of proteinase K (6 mg/ml) were added, and the mixture was incubated at 37°C for 1 hr. Half a volume of phenol saturated with 0.1 M Tris buffer (pH 8.0) and half a volume of chloroform/isoamyl alcohol (24：1) mixture were added, mixed gently, and centrifuged for 15 min at 10,000×*g*. The aqueous phase was collected, and sodium acetate was added at a final concentration of 0.3 M. Following the addition of 0.6 volume of isopropanol, the DNA was collected by centrifugation at 10,000×*g* for 20 min and suspended in 2 ml of sterile distilled water. RNase A was added at a final concentration of 10 μg/ml and incubated at 37°C for 30 min. The phenol/chloroform/isoamyl alcohol extraction was carried out as described above. The aqueous phase collected was dialyzed against TE buffer. Unrestricted DNA and restriction enzyme-digested DNA were electrophoresed in 1% agarose gels and transferred to nitrocellurose membranes (Hybond-ECL, Amersham). Labelling of Xf2 RF-DNA (10 ng/μl) with horseradish peroxidase, hybridization and detection by exposure on autoradiography film (Hyperfilm-ECL, Amersham) were performed using an ECL direct nucleic acid labelling and detection kit (Amersham), according to the manufacturer's instructions.

The total DNA isolated from each *X. c.* pv. *oryzae* strain was probed with Xf2 RF-DNA (Fig. 1). The probe RF-DNA hybridized with the DNA isolated from N5845 and N5875, not with the DNA isolated from uninfected N5850 (Fig. 1, lane 3′, 4′, 5′). The hybridized bands were observed only in the position of bacterial chromosomal DNA. The results indicated that the Xf2 genome was inserted into the chromosomal DNA of the strains N5845 and N5875. The total DNA isolated from Xf2-infected bacteria contained three kinds of free forms of Xf2 genome (open circular RF-DNA, supercoiled RF-DNA, and single-stranded DNA) and bacterial chromosomal DNA (Fig. 1, lane 2). Hybridization occurred with the three forms of Xf2 DNA, but not with chromosomal DNA (Fig. 1, lane 2′). The electrophoretic mobility of the lowest band (Fig. 1, lane 2) was the same as single-stranded Xf2 DNA extracted from partially purified Xf2, and the lowest band was hybridized with a Xf2 DNA probe. Furthermore, the lowest band was dyed dark orange with acridine orange (30 μg/ml)[7]. This showed that the DNA was single-stranded. From these facts, it was concluded that the lowest band was single-stranded Xf2 DNA. No free forms of Xf2 DNA could be detected in the DNA samples isolated from N5845 and N5875 by Southern blot analyses (Fig. 1, lane 4′, 5′). This indicated that a negligible amount of the free Xf2 genome was present in these DNA samples.

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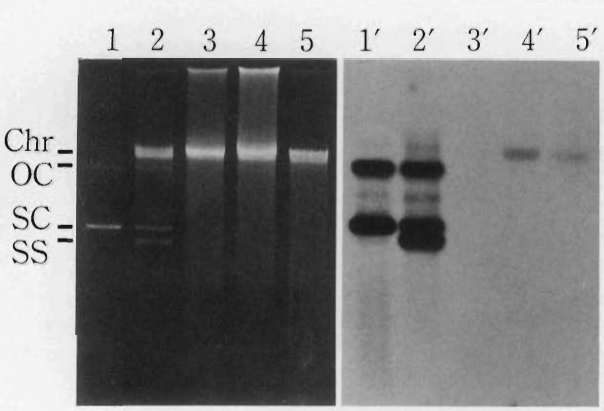

Fig. 1. Southern hybridization analysis of total DNA from *X. campestris* pv. *oryzae*. (Left panel) 1% agarose gel stained with ethidium bromide. lane 1, Xf2 RF-DNA; lane 2, total DNA from indicator strain N5850 infected with Xf2 phage; lane 3, total DNA from indicator strain N5850; lane 4, total DNA from lysogen N5845; lane 5, total DNA from lysogen N5875; Chr, chromosomal DNA; OC, open circular RF-DNA; SC, supercoiled RF-DNA; SS, single-stranded Xf2 DNA. (Right panel) Southern blot of the gel in the left panel probed with labeled Xf2 RF-DNA.

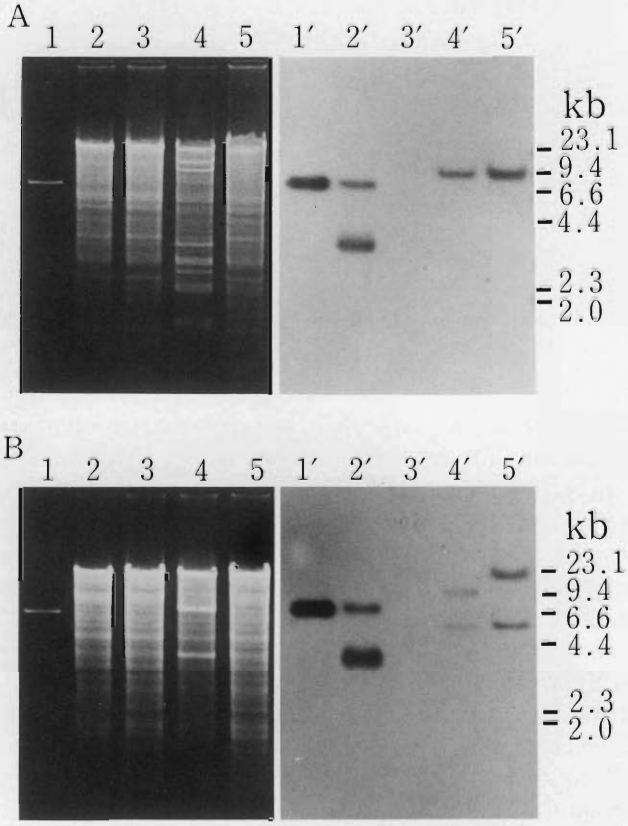

Fig. 2. Southern hybridization analysis of total DNA from *X. campestris* pv. *oryzae*. All DNA samples were digested with *Bam*HI(A) or *Eco*RI(B). (left panels) 1% agarose gels stained with ethidium bromide. lane 1, Xf2 RF-DNA; lane 2, total DNA from indicator strain N5850 infected with Xf2 phage; lane 3, total DNA from indicator strain N5850; lane 4, total DNA from lysogen N5845; lane 5, total DNA from lysogen N5875. (Right panels) Southern blots of the gels in the left panels probed with labeled Xf2 RF-DNA. The positions of size markers in the kilobase pairs are shown in the right margin. Lambda DNA digested with *Hind*III was used.

For further confirmation of the results shown in Fig. 1, the total DNA samples were digested with *Bam*HI or *Eco*RI. *Bam*HI cleaved RF-DNA at one site to yield a 7.2 kb fragment[4] (Fig. 2A, lane 1). *Eco*RI cleaved RF-DNA at two sites to yield two fragments (6.82 and 0.38 kb)[4]. The 6.82 kb fragment was detected (Fig. 2B, lane 1); however, the 0.38 kb fragment was not detected because of its small size. When the total DNA samples from N5845 and N5875 were digested with *Bam*HI or *Eco*RI, the RF-DNA probe hybridized with one or two junction fragments consisting of phage and host DNA (Fig. 2A and 2B, lane 4′, 5′). No hybridization band was detected in the N5850 DNA digested with *Bam*HI or *Eco*RI (Fig. 2A and 2B, lane 3′). When the total DNA from N5850 infected with Xf2 was digested with the same restriction enzymes, hybridization of the RF-DNA probe occurred with linear Xf2 RF-DNA (upper band) and single-stranded Xf2 DNA (lower band), but without any fragments (Fig. 2A and 2B, lane 2′). The above results confirmed that the Xf2 DNA was integrated into the chromosomal DNA of N5845 and N5875.

The present study demonstrates that *X. c.* pv. *oryzae* N5845 and N5875 lysogenize with filamentous phage Xf2. However, the lysogenic filamentous phage Xf2 in the two strains was not induced by mytomycin C treatment (0.005, 0.1 and 1 μg/ml). It was also discovered that free forms of Cf16 DNA can coexist with the integrated form of Cf16 DNA[1]. These properties of the filamentous phage lysogen seem to be fairly different from those of the lysogen of tadpole phage, such as lambda phage. Therefore, further studies of lysogenization and induction of Xf2 are needed to reveal its life cycle.

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和 文 摘 要

上運天博：繊維状ファージ Xf2 DNA の宿主染色体 DNA への組み込み

Xf2 ファージ産生菌である *Xanthomonas campestris* pv. *oryzae* N5845 および N5875 から全 DNA を抽出し，セイヨウワサビ・ペルオキシダーゼで直接標識した Xf2 の複製型(RF) DNA をプローブとしてサザンハイブリダイゼーションを行った。その結果，N5845 および N5875 の染色体 DNA とハイブリッドを形成した。さらに全 DNA を *Bam*HI または *Eco*RI で消化した後，ハイブリダイゼーションを行った結果，Xf2 DNA と宿主細菌の染色体 DNA から成る断片とハイブリッドを形成した。以上の結果より，Xf2 ファージ産生菌の N5845 および N5875 の染色体 DNA に Xf2 DNA が組み込まれていることが明らかとなった。

(Received October 20, 1994 ; Accepted December 27, 1994)