Selective Induction of Two Temperate Phages in Bacillus thuringiensis Strain AF101

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Bacillus thuringiensis is well known for the production of parasporal inclusions (crystals) toxic for lepidopterous, dipterous and coleopterous insects. B. thuringiensis strain AF101, belonging to H-serotype 4a:4b, produces crystals which are slightly toxic for the silkworm, Bombyx mori, but highly toxic for the common cabbageworm, Pieris rapae crucivora, the fall webworm, Hyphantria cunea, and the diamondback moth, Plutella xylostella.1) This strain is advantageous for use for microbial control of insect pests in sericultural areas. In this paper, we describe the selective induction of two temperate phages, J7W-1 and KK-88, in B. thuringiensis strain AF101.

Phage induction with ethidium bromide as an intercalating agent for DNA molecules2,3) was performed by cultivating cells of B. thuringiensis strain AF101 at 27°C in LB broth4) containing 1 μg ethidium bromide. Cell lysis usually occurred after culturing for about 6 hr. The induction of phage by UV irradiation was attempted as follows: cells cultured until the mid-log phase were suspended in PBS (10 mM phosphate buffer containing 0.7% NaCl, pH 7.0) and then 10 ml of the cell suspension was transferred to a petri dish (90 mm in diameter). Cells were exposed to UV irradiation for 30 sec, using a 15 W Toshiba germicidal lamp (model GL-1) at the distance of 50 cm. After UV irradiation, the cells were inoculated into fresh LB broth and cultured at 27°C for about 5 hr, when cell lysis reached the maximum level. A phage suspension was prepared from each cell lysate by centrifugation at 20,000 × g for 1 hr at 4°C, after removing cell debris by centrifugation at 10,000 × g for 20 min at 4°C. Phage particles were purified by 10~40% sucrose density gradient centrifugation in a Spinco SW25-1 rotor at 33,000 rpm for 1 hr at 4°C.

In strain AF101, two temperate phages were induced by two inducers. Phage J7W-1 induced by ethidium bromide treatment has a hexagonal head (65 nm in diameter) and a tail (290 nm), but phage KK-88 induced by UV irradiation is slightly smaller than J7W-1 (head, 49 nm in diameter, and tail length, 200 nm) (Fig. 1). The two phages were different from other temperate phages hitherto reported in B. thuringiensis in the shape of the head and the tail.

Fig. 1. Electron Micrograph of Phages J7W-1 (A) and KK-88 (B).
The samples were stained with 1% uranyl acetate. Bars indicate 50 nm.

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Fig. 2. Phage Induction by Ethidium Bromide (A) and UV Irradiation (B) in B. thuringiensis Strain AF101.
The optical densities of normal (○) and treated cell (●) cultures were measured at OD 660 nm. The number of
plaque forming units (PFU) of phages was determined by using the type strain of subsp. israelensis as the
indicator of phages (■).

Fig. 3. Comparison of Restriction Patterns in J7W-1 DNA (A) and KK-88 DNA (B).
The size markers (in kb) in the right margin are the HindIII fragments of lambda phage DNA.

References