MATING IN *BACILLUS THURINGIENSIS* CAN INDUCE PLASMID INTEGRATIVE PROPHAGE J7W-1

K. KANDA*, Y. TAKADA, F. KAWASAKI, F. KATO, A. MURATA

Institute of Applied Microbiology, Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Saga 840-8502, Japan

Received March 3, 2000; accepted July 3, 2000

Summary. – *Bacillus thuringiensis* serovar *israelensis*, a bacterium which possesses plasmid transfer ability after mating, has been lysogenized by plasmid integrative phage J7W-1. The induction of phage in this J7W-1 lysogen was observed after mating with phage-insensitive strains, such as *B. thuringiensis* serovar *thuringiensis, B. cereus* and *B. subtilis*, as well as the phage-sensitive strain serovar *israelensis*. The phage induction was not observed after mating with *B. thuringiensis* strains AF101, serovar *dendrolimus* and serovar *indiana*. Because these strains are naturally associated with J7W-1 or its related phage, the data strongly suggest a constitutive expression of the repressor encoded by the prophage in these strains. However, the phage induction was observed in *B. thuringiensis* serovar *aizawai*, although it contained the J7W-1 DNA homologous region(s).

Key words: plasmid integrative prophage; phage induction by mating; Bacillus thuringiensis serovar israelensis

Introduction

The plasmid integrative phage J7W-1 from *B. thuringiensis* strain AF101 is a phage that is readily inducible by ethidium bromide treatment but not by other commonly used methods such as UV irradiation (Kanda *et al.*, 1989; Kanda and Aizawa, 1989). The mechanism of phage induction in the lysogen appears to be mediated via a cellular SOS response triggered by damaged cellular DNA. A proteolytic DNA repair enzyme induced by a SOS response cleaves the repressor encoded by the phage and then the lytic growth of the prophage is initiated in the lysogen (Friedman and Gottesman, 1983; Love and Yasbin, 1984). Accordingly, we postulate that the ethidium bromide treatment can induce the phage from the lysogenic strain, since this molecule intercalates the hydrogen bonds in DNA and inhibits DNA synthesis *in vivo* (Bouanchaud *et al.*, 1968). Although various treatments have been shown to induce temperate phages in *B. thuringiensis* (Colasito and Rugoff, 1969; Affray and Boutibonnes, 1985; Reynolds *et al.*, 1988; Inal *et al.*, 1990), the induction of phage by the ethidium bromide treatment has not, to our knowledge, been reported (except in the case of J7W-1). Therefore, elucidation of the mechanism of the ethidium bromide-induced phage induction in *B. thuringiensis* may reveal new SOS systems in this bacterium, perhaps revealing the expression of novel proteolytic DNA repair enzyme(s).

We have reported earlier on the distribution of ethidium bromide-inducible phage, J7W-1 and/or its related phage, in *B. thuringiensis* strains (Kanda *et al.*, 1998). We also identified three types of bacterial strains naturally associated with J7W-1, J7W-related phage, or just the homologous region(s) of the phage genome (Kanda *et al.*, 1998). It is hoped that the use of these strains will provide the key needed to open the door in our understanding of the ethidium bromide induction. To this end, we have observed that mating in *B. thuringiensis* serovar *israelensis* that possesses the plasmid transfer ability can induce the expression of extrachromosomal prophage SU-11 (Kanda *et al.*, 1999). This observation indicates that the J7W-1 phage induction

*E-mail: kandak@cc.saga-u.ac.jp; fax: +81952-288709.

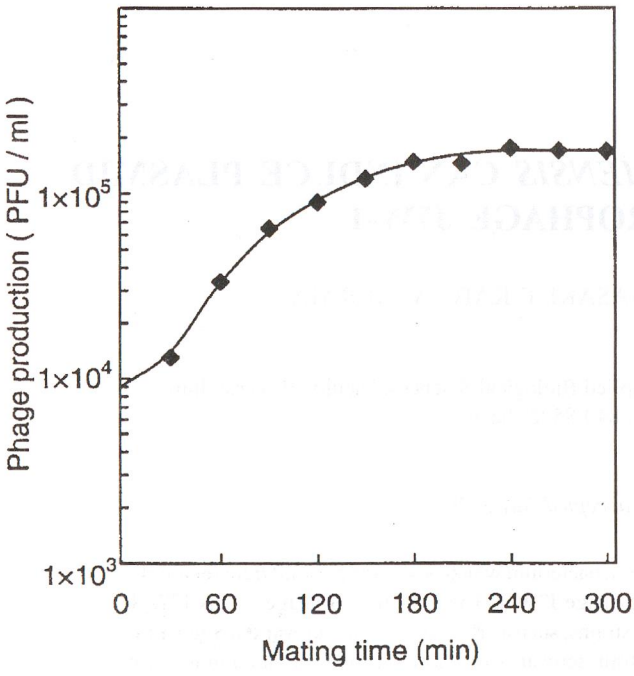

Fig. 1
Phage induction after mating of the J7W-1 lysogen of *B. truringiensis* serovar *israelensis* with the prophage-cured B-29 strain

will occur as a result of mating using J7W-1 lysogenic strain of serovar *israelensis*, since the J7W-1 phage genome is integrated into a plasmid during its lysogenic cycle in the serovar *israelensis* lysogen (Kanda *et al.*, 1989).

In this report, we studied the induction of J7W-1 phage triggered by the mating responses in this bacterium.

Materials and Methods

Bacterial strains. Type strains of *Bacillus thuringiensis* serovars *thuringiensis*, *dendrolimus*, *indiana*, *aizawai* and *israelensis* were obtained from cultures maintained in the Institute of Biological Control, Kyushu University, Fukuoka, Japan. *Bacillus cereus* T and *Bacillus subtilis* Y12S were obtained from the cultures in our laboratory. A prophage-cured strain of serovar *israelensis* (B-29) was prepared as described by Kanda *et al.* (1999) and used as the indicator strain for measuring the phage production. A J7W-1 lysogen of serovar *israelensis* (LIJ-8) was isolated from survival colonies of B-29 after the phage infection.

Culture conditions. The bacteria were routinely grown in LB broth (pH 7.0) supplemented with Bacto trypton (1% w/v), yeast extract (0.5% w/v) and NaCl (1% w/v). LB broth containing 1.5% (w/v) agar was used for the solid agar medium. All bacterial cultures were incubated at 27°C, except for *B. subtilis* Y12S that was incubated at 37°C.

Mating conditions. The prophage donor strain LIJ-8 and each recipient strain were allowed to grow until the mid-log phase. Equal number of donor and recipient cells (2.0×10^6) were then mixed in 2 ml of LB broth. The mixture was incubated at 27°C with gentle shaking in order to allow for mating to occur. During the experiment, 200 µl aliquots of the mixture were collected at 30 mins intervals and passed through a Millex GV4 filter (0.22 µm, Millipore) to remove the cells. The titer of the mating mixture was assayed using B-29 as an indicator strain and was expressed in PFU/ml.

Results and Discussion

It was observed that phage induction frequently occurred after mating LIJ-8 as J7W-1 lysogen with the phage-cured strain B-29, a strain sensitive to J7W-1 infection (Fig. 1). However, the spontaneous phage induction was not observed during cultivation of the lysogen without mating. The production of phage was found to increase along the mating time and it reached the maximum level (1.7×10^5 PFU/ml) at 150 mins after mating. The plaque forming phage observed on the LB agar plates after mating was identified as J7W-1 using a plaque hybridization technique with J7W-1 DNA as the probe (data not shown). The induction of J7W-1 phage was observed after mating LIJ-8 not only with B-29, but also with the J7W-1-insensitive strains, such as *B. thuringiensis* serovar *thuringiensis*, *B. cereus* and *B. subtilis* (Fig. 2). The phage production after mating with those phage-insensitive strains reached the maximum level of about 2.0×10^5 PFU/ml. The process of J7W-1 or SU-11 phage induction by mating appears to occur by similar mechanisms. It is notable, however, that there were significant differences in the time required to reach maximum phage production levels by the strains tested: i.e. J7W-1 took 120–180 mins while SU-11 took only 40 mins.

Because the phage induction observed as a result of mating was identified to occur in strains beyond the host range for J7W-1, we then examined the phage induction by mating LIJ-8 with lysogens of J7W-1 or its related phage (strain AF101, serovar *dendrolimus* and serovar *indiana*). The serovar *aizawai* was also included in this study as it possessed homologous region(s) to J7W-1 DNA on the plasmid. The results of these experiments indicated that while phage induction clearly took place as a result of LIJ-8 mating with serovar *aizawai*, no phage induction was observed in recipient strains of AF101, serovars *dendrolimus* and *indiana* which were either associated with J7W-1 or its related phage (Fig. 3). Since the phage induction by mating may be due to dilution of repressors within a recipient cell, this phenomenon obviously indicated that the expression of transferred phage genome was inhibited either by a repressor protein produced in the lysogens of J7W-1 or its related phage. In the case of serovar *indiana*, we have postulated

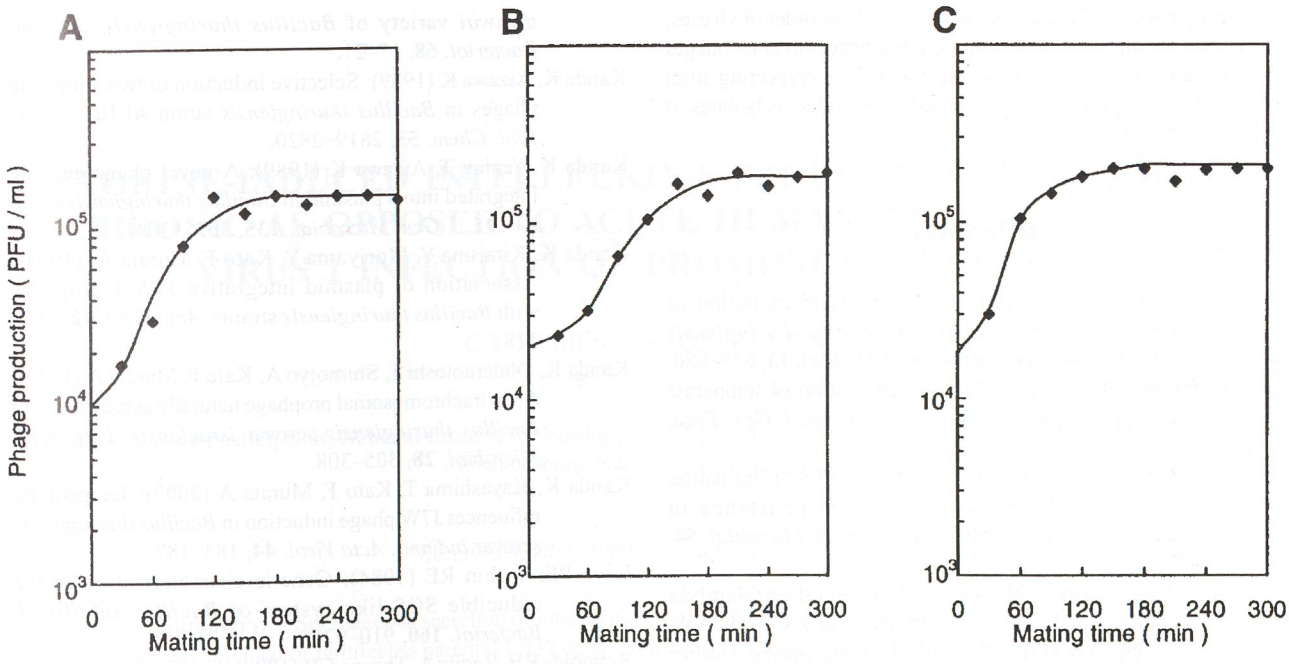

Fig. 2
Phage induction after mating of the J7W-1 lysogen of *B. truringiensis* serovar *israelensis* with J7W-1-insensitive bacteria belonging to the genus *Bacillus*
B. thuringiensis serovar *thuringiensis* (A), *B. cereus* (B), and *B. subtilis Y12S* (C).

that the phage induction in this strain is regulated by the same repressor mechanism observed for J7W-1 prophage, although the phage production by the ethidium bromide treatment is low compared to that found in strain AF101, J7W-1 lysogen (Kanda *et al.*, 1998, 2000). The results obtained in this study strongly support such a suggestion. On the other hand, it was revealed that serovar *aizawai* did not produce the repressor to regulate the phage induction. Thus, it is also possible that the repressor gene may not be located in the homologous region to J7W-1 genome identified in the plasmid in this bacterium.

In conclusion, we found that J7W-1 phage can be induced after mating has taken place using its lysogenic strain of serovar *israelensis* as a donor of the phage genome. Since the phage genomes can be transferred into well characterized bacterial strains, such as *B. subtilis*, it is hoped that the use of mating systems will shed new light into the mechanism of ethidium bromide phage induction.

Acknowledgement. The authors thank Dr. D.J. Grab, Saga University, Japan, for helpful discussion and help in preparing our manuscript.

Note of the Editor-in-Chief. As the J7W-1 and J7W-1-related phages described in this paper were so far not accepted by the International Committee on Taxonomy of Viruses (ICTV) as new

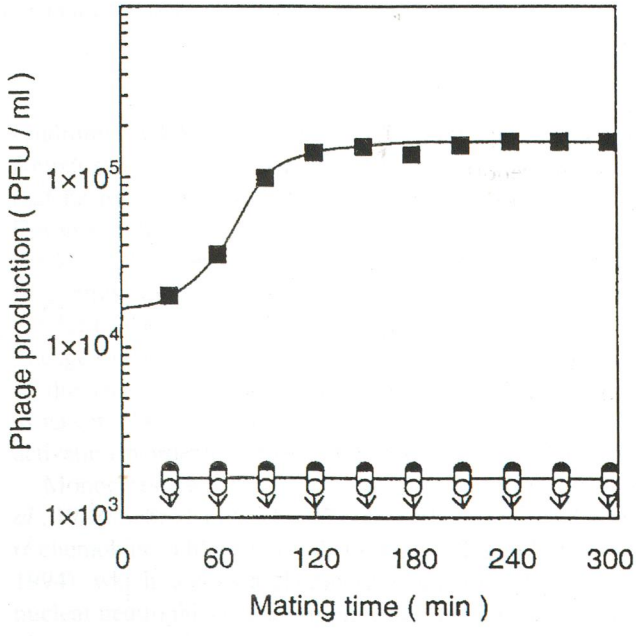

Fig. 3
Phage production after mating of the J7W-1 lysogen of *B. truringiensis* serovar *israelensis* with *B. thuringiensis* strains naturally associated with the J7W-1 genome
AF101 (open circles), serovar *dendrolimus* (open squares), serovar *indiana* (closed circles), and serovar *aizawai* (closed squares).

members (species) of any known genus, family or order of viruses, (1) the idendity and relationship of these two phages to other phages so far recognized by ICTV are unclear and (2) in respecting strict rules of ICTV these two phages should be regarded as isolates of so far unknown phages (species).

References

Affray Y, Boutibonnes P (1985): Discriminated induction of prophages and bacteriocin in *Bacillus thuringiensis* strains by genotoxic agents. *IRCS Med Sci.* **13**, 679–680.

Colasito DJ, Rogoff MH (1969): Characterization of temperate bacteriophages of *Bacillus thuringiensis*. *J. Gen. Virol.* **5**, 275–280.

Bouanchaud DH, Scavizzi MR, Chabbert YA (1968): Elimination by ethidium bromide of antibiotic resistance in enterobacteria and staphylococci. *J. Gen. Microbiol.* **54**, 417–425.

Friedman D, Gottesman M (1983): Lytic mode of lambda development. In Hendrix RW, Roberts JW, Stahl FW, Weisberg RA (Eds): *Lambda II.* Cold Spring Harbor Laboratory, Cold Spring Harbor, pp. 21–51.

Inal JRM, Karunakaran V, Burges HD (1990): Isolation and propagation of phages naturally associated with the *aizawai* variety of *Bacillus thuringiensis*. *J. Appl. Bacteriol.* **68**, 17–21.

Kanda K, Aizawa K (1989): Selective induction of two temperate phages in *Bacillus thuringiensis* strain AF101. *Agric. Biol. Chem.* **53**, 2819–2820.

Kanda K, Yeping T, Aizawa K (1989): A novel phage genome integrated into a plasmid in *Bacillus thuringiensis* strain AF101. *J. Gen. Microbiol.* **135**, 3035–3041.

Kanda K, Kitajima Y, Moriyama Y, Kato F, Murata A. (1998): Association of plasmid integrative J7W-1 prophage with *Bacillus thuringiensis* strains. *Acta Virol.* **42**, 315–318.

Kanda K, Ohderaotoshi T, Shimojyo A, Kato F, Murata A (1999): An extrachromosomal prophage naturally associated with *Bacillus thuringiensis* serovar *israelensis*. *Lett. Appl. Microbiol.* **28**, 305–308.

Kanda K, Kayashima T, Kato F, Murata A (2000): Temperature influences J7W phage induction in *Bacillus thuringiensis* serovar *indiana*. *Acta Virol.* **44**, 183–187.

Love PE, Yasbin RE (1984): Genetic characterization of the inducible SOS-like system of *Bacillus subtilis*. *J. Bacteriol.* **160**, 910–920.

Reynolds RB, Reddy A, Thorne CB (1988): Five unique temperate phages from a polylysogenic strain of *Bacillus thuringiensis* subsp. *aizawai*. *J. Gen. Microbiol.* **134**, 1577–1585.