TEMPERATURE INFLUENCES INDUCTION OF A J7W-1-RELATED PHAGE IN *BACILLUS THURINGIENSIS* SEROVAR *INDIANA*

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Summary. – Induction of a plasmid-integrative J7W-1-related phage *in Bacillus thuringiensis* serovar *indiana by* ethidium bromide was influenced by the temperature at which the host cells were cultured. Under optimal growth conditions, the maximum titer of the phage produced by the serovar *indiana* reached 1.2 x 10^6 PFU/ml at 37°C while at 27°C it was lower by an order of magnitude (1.3 x 10^5 PFU/ml). The temperature-sensitive period was estimated to occur early during the phage induction. However, the temperature effect observed with the serovar *indiana* did not occur with the serovar *israelensis.* In the latter case, the phage induction was the same at 37°C or 27°C. Thus we assume that the temperaturesensitive phage induction observed with the serovar *indiana* as host was not a phenomenon caused by the phage genome but rather by product(s) encoded by certain host gene(s).

Key words: Bacillus thuringiensis; plasmid-integrative phage; J7W-1-related phage; ethidium bromide induction; temperature sensitivity

Introduction

B. thuringiensis is a bacterium with well known insecticidal properties. The ability of this bacterium to produce endotoxins highly toxic for *lepidopteran, dipteran* and *coleopteran* insect larvae but harmless for vertebrates is well established. Because of its agricultural importance, genetic studies aimed at improving this bacterium's ability to act as an effective microbial pest control agent are clearly desirable.

The induction of temperate phages using various experimental methods has been reported for several strains of *B. thuringiensis* (Colasito and Rogoff, 1969; Affray and Boutibonnes, 1985; Reynolds *et al.*, 1988; Inal *et al.*, 1990), and it has been suggested to use the phage genes related to the induction for construction of new expression vectors containing a genetic switch. While the phage induction is known to require an intimate interplay of genetic functions of the phage and the host bacterium, little attention has been so far paid to the mechanism of phage induction in *B. thuringiensis.* By analyzing the requirements for phage induction in these systems it is anticipated that not only will we discover new temperate phages, but we will also learn how to make use of this interesting class of viruses as genetic tools to analyze and/or improve *B. thuringiensis* strains as a beneficial agricultural commodity.

It has been a decade since we described the isolation of the plasmid-integrative phage J7W-1 in *B. thuringiensis* strain AF101 (Kanda *et al.*, 1989). This phage was inducible by ethidium bromide but not by conventional ultraviolet irradiation in this host (Kanda and Aizawa, 1989). Accordingly, a novel mechanism for J7W-1 prophage induction was suggested. Recently, we observed that a J7W-related prophage was naturally associated with *B. thuringiensis* serovar *indiana.* Unlike the J7W-1 prophage, the J7W-1-related prophage, although ethidium bromide-inducible, was not inducible to the same level as JW7-1 prophage in *B. thuringiensis* strain AF101 (Kanda *et al.*, 1998). Because of these observations we have further investigated the mechanism of phage induction by ethidium

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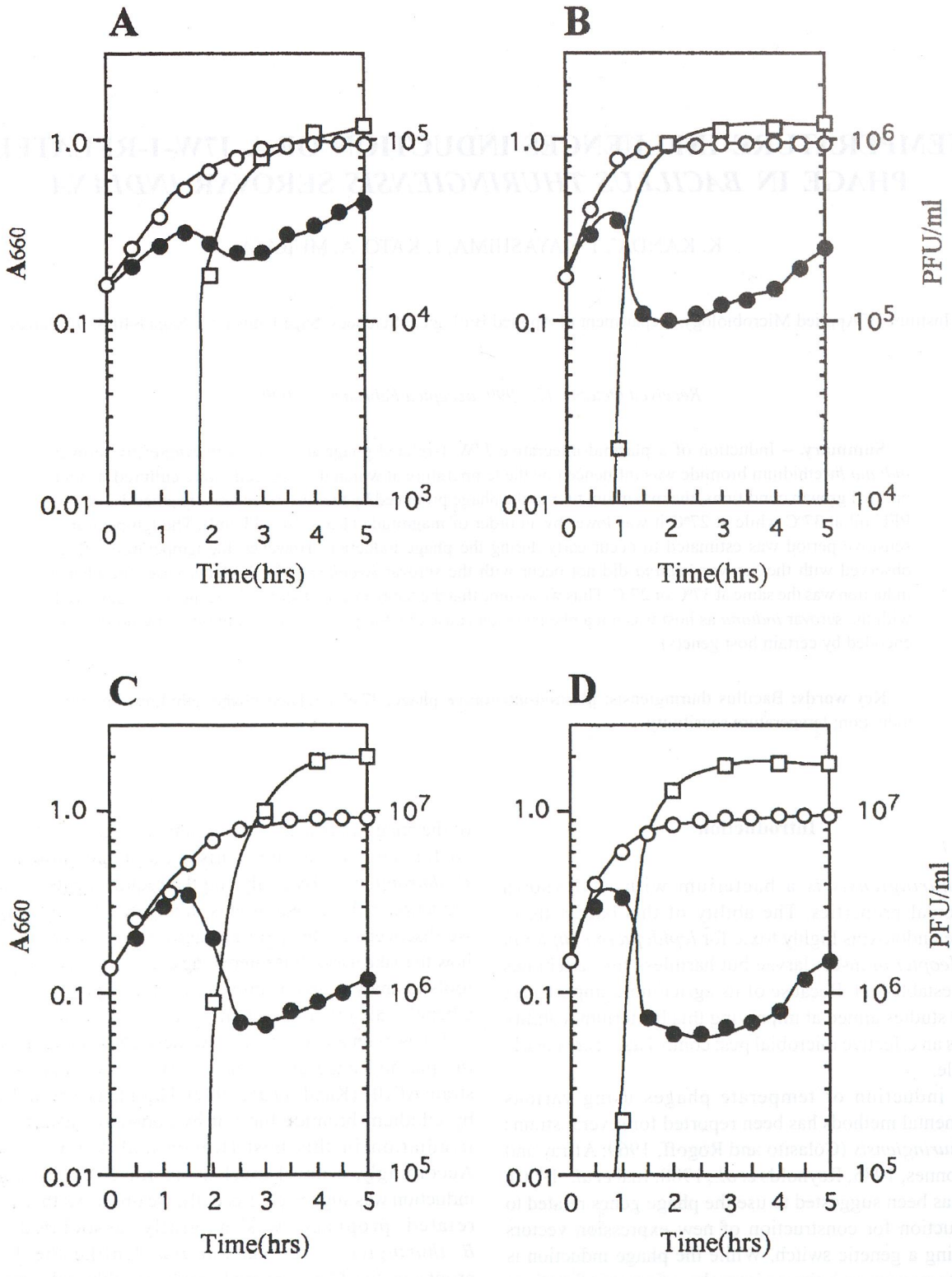

Fig. 1

Effect of temperature on phage induction by ethidium bromide in *B. thuringiensis* serovar *indiana* and AF101 strain

Serovar *indiana* (A and B) and AF101 strain (C and D). 27°C (A and C) and 37°C (B and D). The induced (full circles) and non-induced (empty circles) cultures. Phage titer (PFU/ml, empty squares).

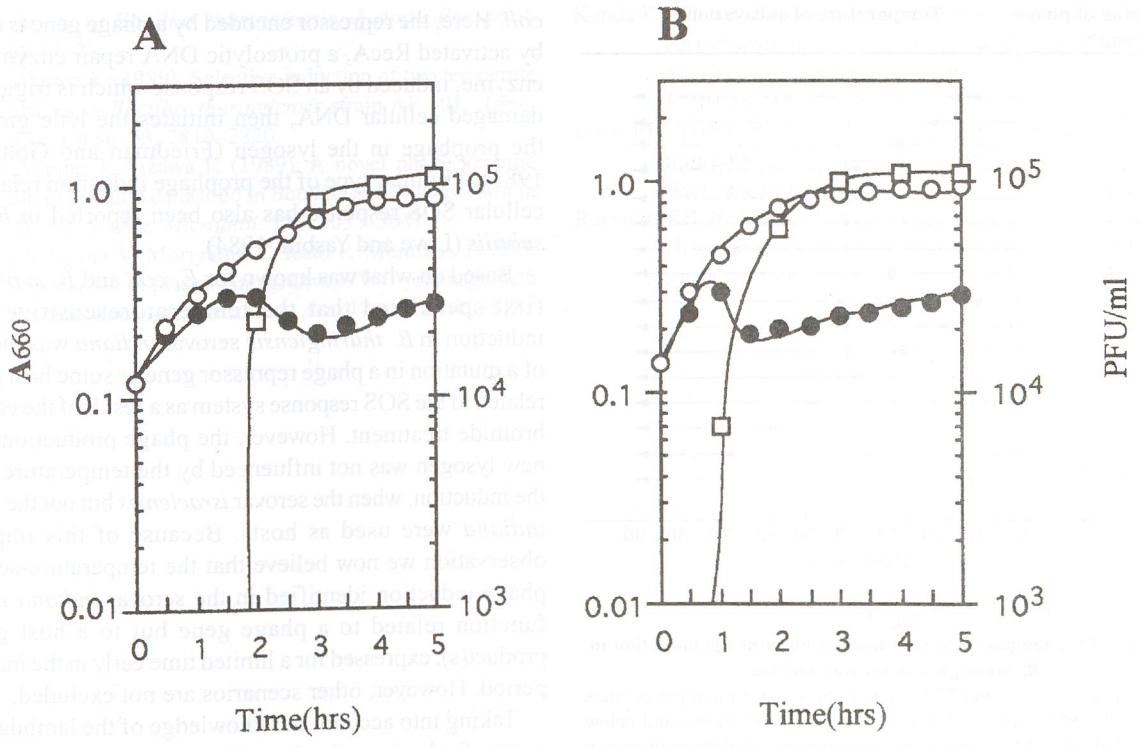

Fig. 2

Effect of temperature on phage induction by ethidium bromide in *B. thuringiensis* serovar *israelensis* lysogens prepared by use of the phage induced from serovar *indiana* 27°C (A) and 37°C (B)

The induced (full circles) and non-induced (empty circles) cultures. Phage titer (PFU/ml, empty squares).

bromide. We now report our findings describing the influence of the temperature of host cell cultivation on the phage induction by ethidium bromide.

Materials and Methods

Bacterial strains. *B. thuringiensis* strain AF 101 (J7W-1 lysogen), serovars *indiana* and *israelensis*, were obtained from the Institute of Biological Control, Kyushu University, Kyushu, Japan. The lysogenic strain of serovar *israelensis* was prepared from the colonies which survived after the phage infection.

Culture conditions. The cultivation of *B. thuringiensis* strains was usually carried out at 27°C. The influence of the temperatures of 27°C and 37°C on phage induction in the lysogenic strains was tested. The bacteria were grown in LB broth pH 7.0 containing 10 g/l of Bacto-trypton, 5 g/l yeast extract, and 10 g/l NaCl. For the solid medium, 1.5% of agar was added prior to sterilization.

Phage induction. The induction of phage by ethidium bromide was carried out as described previously (Kanda *et al.*, 1989). Phage production was measured using the indicator strain B29, a prophage-cured strain of *B. thuringiensis* serovar *israelensis* (Kanda *et al.*, 1999).

Results

The production of the phage after the ethidium bromide induction at 27°C was approximately by one order lower in the serovar *indiana* (1.3 x 10^5 PFU/ml at maximum) than in AF 101 strain (2.0 x 10^7 PFU/ml at maximum) (Fig. 1a, c). However, the phage production in the serovar *indiana* increased markedly reaching a maximum level of 1.2 x 10^6 PFU/ml, at the induction temperature increased to 37°C (Fig. 1b). In contrast, we found no significant effects of these temperatures on the phage induction in AF101 strain (Fig. 1c, d). A lysogenic strain of serovar *israelensis* of the JW7-1-related phage induced from serovar *indiana* was subsequently prepared and the phage induction was examined in this lysogen again at 27°C and 37°C. As shown in Figs. 2a and 2b, the phage production in the lysogen was almost identical at either induction temperature. Concerning the J7W-1 lysogen of serovar *israelensis*, the phage production at 27°C or 37°C was similar in *B. thuringiensis* strain AF 101. Taken together, these facts suggest that the temperature sensitivity of the phage induction by ethidium bromide in the serovar *indiana* was not caused by the phage genome but by the host chromosome.

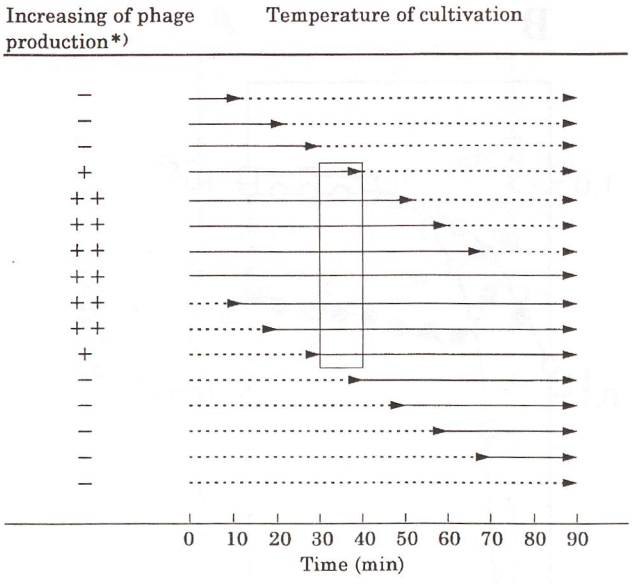

Fig. 3
Detection of the temperature-sensitive period of phage induction in *B. thuringiensis* serovar *indiana*
37°C (continuous lines) and 27°C (dotted lines). Maximum phage titers over 1.0 x 10^6 PFU/ml (++), 2.0 x 10^5–1.0 x 10^6 PFU/ml (+), and below 2.0 x 10^5 PFU/ml (–) The temperature-sensitive period of phage induction is marked by the open square.

Influence of temperature on the production of phage in the serovar *indiana* was, therefore, analyzed further by shifting the cultivation temperature from 27°C to 37°C *or vice versa* at 10 mins intervals after the phage induction. The results of these experiments are shown in Fig. 3. When the temperature was shifted from 37°C to 27°C, the effect of the higher temperature was terminated 30 mins after the induction at 37°C. On the contrary, increasing the temperature from 27°C to 37°C stopped the phage production 40 mins after the induction at 27°C. Thus it appears that the influence of temperature on phage production occurs within a narrow period of 30–40 mins after the induction. Furthermore, this period also coincided with the early stage of the induction when there was no phage production.

Discussion

The phage induction by ethidium bromide has been so far observed only in *B. thuringiensis* strains (Kanda *et al.*, 1989, 1998). In identifying the molecular mechanism of the ethidium bromide induction we discovered that the phage induction in *B. thuringiensis* serovar *indiana is* a temperature-sensitive phenomenon.

The mechanism of phage induction in lysogen has been fully explained in the case of lambda phage in *Escherichia coli*. Here, the repressor encoded by a phage gene is cleaved by activated RecA, a proteolytic DNA repair enzyme. This enzyme, induced by an SOS response which is triggered by damaged cellular DNA, then initiates the lytic growth of the prophage in the lysogen (Friedman and Gottesman, 1983). A similar type of the prophage induction related to a cellular SOS response has also been reported *in Bacillus subtilis* (Love and Yasbin, 1984).

Based on what was known for *E. coli* and *B. subtilis,* we first speculated that the temperatureresensitive phage induction in *B. thuringiensis* serovar *indiana* was the result of a mutation in a phage repressor gene or some host gene(s) related to the SOS response system as a result of the ethidium bromide treatment. However, the phage production in this new lysogen was not influenced by the temperature during the induction, when the serovar *israelensis* but not the serovar *indiana* were used as hosts. Because of this important observation we now believe that the temperature-sensitive phage induction identified in the serovar *indiana* is not a function related to a phage gene but to a host gene(s) product(s), expressed for a limited time early in the induction period. However, other scenarios are not excluded.

Taking into account our knowledge of the lambda phage system further investigation of (1) the host gene(s) product(s) of concern and (2) the host SOS response system is required.

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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 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).

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