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Immunological Relationship of Crystal Proteins of Six *Bacillus thuringiensis* Serovarieties; *B. thuringiensis* serovar. *coreanensis* (H25), *konkukian* (H34), *leesis* (H33), *seoulensis* (H35), *sooncheon* (H41) and *yosoo* (H18a18c)

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Abstract Crystals of six new *Bacillus thuringiensis* serovarieties [*coreanensis* (H25), *konkukian* (H34), *leesis* (H33), *seoulensis* (H35), *sooncheon* (H41), and *yosoo* (H18a18c)] with different H-antigens, which are toxic to *Bombyx mori* and/or mosquito larvae, were serologically quite distinct from each other.

Key words: *Bacillus thuringiensis* serovar. *coreanensis*, *konkukian*, *leesis*, *seoulensis*, *sooncheon*, and *yosoo*

Six new serovarieties of *Bacillus thuringiensis* with different H-antigens, which are toxic to *Bombyx mori* and mosquito larvae, have been reported and designated as follows: *B. thuringiensis* serovar. *coreanensis* (H25), *konkukian* (H34), *leesis* (H33), *seoulensis* (H35), *sooncheon* (H41), and *yosoo* (H18a18c) [4, 6, 7]. The toxicity to mosquito and *B. mori* larvae is caused by the parasporal crystal, which induces paralysis in mid-gut [3, 5]. Crystal serology of *B. thuringiensis* has been shown to correlate with toxicity spectra of *B. thuringiensis* strains and should be considered to be an important taxonomic identity [9]. Therefore, the immunological relationships of six new *B. thuringiensis* serovarieties were investigated.

B. thuringiensis was precultured overnight in 20 ml of LB broth (Difco) at 28°C by 180 rpm rotary agitation, and 1.0 ml of the precultures was transferred into 20 ml of U. G. medium [2]. Then, it was cultured for 6 days until sporulation and lysis at 28°C by 180 rpm rotary agitation. Formation of spores and parasporal crystals was observed with a phase contrast microscope. The fully matured crystals and lysed cells were harvested and washed twice with sterilized H_2O by centrifugation at 12,000 ×*g* for 10 min. The crystal and spore mixtures were incubated overnight in 0.01% Triton X-100 and 1 M NaCl solution at 4°C. Parasporal crystals were separated from spores and cellular debris by 30 to 70% NaBr gradient centrifugation at 17,000 ×*g* for 120 min [8]. After centrifugation, the crystal layer (upper layer) was harvested with pasteur pipette, dissolved in H_2O, and washed twice by centrifugation. The crystal pellets in H_2O were stored at -20°C.

Parasporal crystals of *B. thuringiensis* were separated by NaBr gradient centrifugation [8], and dissolved in alkaline solution and dialyzed [9]. The purified proteins were used for antiserum preparation. Antisera were prepared in rabbits by four weekly subcutaneous injections of approximately 1 mg of the solubilized crystal protein antigen in complete Freund's adjuvant. One week after the last injection, they received a booster injection of the same antigen mixed with Freund's imcomplete adjuvant. The rabbits were bled one week thereafter. Antisera were inactivated by heating at 56°C for 30 min and then stored at -20°C [1, 9].

Fig. 1. Immunodiffusion analysis of crystal proteins from *B. thuringiensis* serovarieties.
Ten μl of antiserum of *B. thuringiensis* serovar. *konkukian* in the center well (C) were allowed to cross-react with the wells around the C well containing 10 μl of antigens of *B. thuringiensis* serovarieties; a, *konkukian*; b, *leesis*; d, *coreanensis*; e, *seoulensis*; f, *israelensis*; g, *kurstaki*; h, *yosoo*; and i, *sooncheon*.

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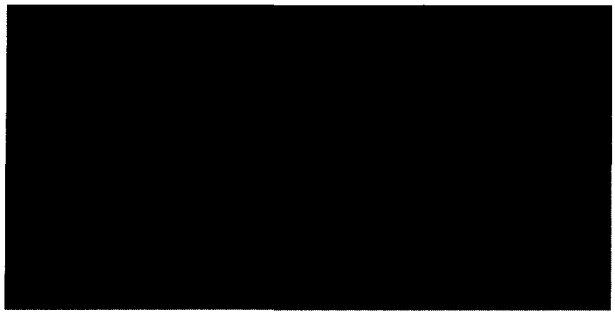

Fig. 2. Immunodiffusion patterns of crystal proteins from *B. thuringiensis* serovarieties.
Ten μl of antiserum of *B. thuringiensis* serovar. *coreanensis* in the center well (C) were allowed to react with the wells around the C well containing 10 μl of antigens of *B. thuringiensis* serovarieties; A, *coreanensis*; B, *seoulensis*; D, *konkukian*; E, *leesis*; F, *yosoo*; G, *sooncheon*; H, *israelensis*; I, *kurstaki*.

Double diffusion was carried out in 1% agarose containing 0.9% NaCl and 0.2% NaN_3 at 4°C for 7 days, and the slides were then washed, dried, and stained with amido black [9].

Ten μl of the antiserum of *B. thuringiensis* serovar. *konkukian* were reacted with the solubilized crystal protein antigens of *B. thuringiensis* serovar. *coreanensis*, *leesis*, *seoulensis*, *sooncheon*, and *yosoo*. A distinct precipitin line was observed only in the homologous system; *konkukian* antigen vs *konkukian* antiserum (Fig. 1). No cross-reactions were observed, when *B. thuringiensis* serovar. *konkukian* antiserum was cross-reacted with crystal antigens produced by serovar. *coreanensis*, *leesis*, *seoulensis*, *sooncheon* and *yosoo*.

Similarly, when 10 μl of the antisera of *B. thuringiensis* serovar. *coreanensis* were reacted with the solubilized crystal protein antigens of *B. thuringiensis* serovar. *konkukian*, *leesis*, *seoulensis*, *sooncheon*, and *yosoo*, distinct precipitin line was observed only in the homologous system; *coreanensis* antigen vs *coreanensis* antiserum (Fig. 2). No cross-reactions were observed when *B. thuringiensis* serovar. *coreanensis* antiserum was allowed to cross-react with crystal antigens produced by serovar. *konkukian*, *leesis*, *seoulensis*, *sooncheon* and *yosoo*.

It is concluded, therefore, that *B. thuringiensis* serovar. *konkukian* and *coreanensis* crystals are serologically quite distinct from those of other *B. thuringiensis* serovarieties which are toxic to lepidoptera or deptera.

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