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REPORT

A cell strain cloned from *Spodoptera exigua* cell line (IOZCAS-Spex-II) highly susceptible to *S. exigua* nucleopolyhedrovirus infection

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Abstract A cell strain (IOZCAS-Spex-II-A) cloned from IOZCAS-Spex-II, a cell line established from the fat body of *Spodoptera exigua* (Lepidoptera: Noctuidae) larva, was characterized, and its capability to produce *S. exigua* nucleopolyhedrovirus was high with infection rate exceeding 90% compared with its parental cell line IOZCAS-Spex-II that scored only 50%. Growth curve of budded virus (BV) in the strain was analyzed and the titer of BV reached the highest of 3.7×10^4 pfu/mL by 96 h after inoculation. Concentration of occlusion bodies (OBs) produced by the cloned cell strain (IOZCAS-Spex-II-A) was 7.1×10^7 OBs/mL, while the parental cell line produced 2.4×10^7 OBs/mL. The average yield of the virus was 176 OBs/cell of IOZCAS-Spex-II-A compared with 211 OBs/cell that of the parental cell line. Significant differences were observed in virus production, growth characters, cell shape, between the parental cell line, and its clone. The cell lines (IOZCAS-Spex-II and IOZCAS-Spex-II-A) were also susceptible to *Autographa californica* multiple nucleopolyhedrovirus infection. In addition, they were characterized with regard to their growth rates and DNA amplification fingerprinting technique employing polymerase chain reaction.

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Cloned insect cell lines with greater susceptibilities to virus replication than their parent lines have been previously reported. Corsaro and Fraser (1987) cloned 24 strains (HZ 1075/UND-A through X) by dilution plating from the established IPLB-HZ 1075 cell line. The capabilities to support replication of *Helicoverpa zea* NPV were different among the cloned cell strains. Lenz et al. (1991) reported that the cloning of *H. zea* cell lines resulted in increased levels of *H. zea* NPV production. Granados et al. (1994) obtained a cell clone named BTI-Tn-5B1-4 which was highly susceptible to *Trichoplusia ni* SNPV with infection rates exceeding 90% by 48 h postinfection (pi). Hara et al. (1993, 1995) produced a cell strain designated Se301, a clone deriving from Se3FH cell line of *Spodoptera exigua*, showing ten times greater sensitivity to *S. exigua* nucleopolyhedrovirus (NPV) compared with the parent cell line.

S. exigua NPV is a very important baculoviral bio-insecticide commercially used for biocontrol of *S. exigua*, a polyphagous pest that infests on a large number of crops all over the world (Vlak et al. 1981; Gelernter et al. 1986; Caballero et al. 1992). It is essential to obtain *S. exigua* NPV susceptible *S. exigua* cell lines or cloned cell strains either for producing the bio-insecticide in vitro or establishing *S. exigua* NPV baculovirus expression vector system (Yasunaga-Aoki et al. 2004). In the present study, one of three daughter cloned strain IOZCAS-Spex-II-A was compared with its parent cell line IOZCAS-Spex-II in cell characters and susceptibility to *S. exigua* NPV and *Autographa californica* multiple nucleopolyhedrovirus (MNPV).

The cell line IOZCAS-Spex-II established from the fat bodies of *S. exigua* larva supported replication of *S. exigua* NPV (Zhang et al. 2006a). The cell line was cultured in TNM-FH medium (Sigma, St. Louis, MO) with 10% inactivated fetal bovine serum in T25-cm^2 tissue culture flasks (Corning, Corning, NY) at 27°C. A 384-well tissue culture plate (Greiner Bio-one, no. 704160) was used to clone the cell. In brief, IOZCAS-Spex-II cells were diluted with a conditioned medium to 16 cells/mL. The conditioned medium was prepared by culturing IOZCAS-Spex-II at a seeding concentration of 10^6 cells/flask (5 mL/flask) at 27°C for 96 h, harvesting the medium, and sterilizing it through a 0.22-μm Millipore filter. One milliliter of the cell suspension in conditioned medium was added to the larger well resulting in an average of one cell in each smaller well. The smaller wells with a single cell 24 h after seeding were marked. After 10 d at 27°C, cell colonies arising from the marked smaller well were selected and moved to a 96-well tissue culture plate with each well containing 50 μL of fresh medium. After reaching confluence, cells were transferred to a 24-well tissue culture plate in 0.5 mL fresh medium. Seven days later, cells that formed a monolayer were then transferred to T25-cm^2 tissue culture flasks containing 4 mL of fresh medium, followed by subculture at 5–7 d intervals.

Three cell clones designated IOZCAS-Spex-II-A, IOZ CAS-Spex-II-B, and IOZCAS-Spex-II-C were successfully isolated and grew to confluency in T25-cm^2 tissue culture flasks about 2 mo after starting the clone procedure. Cells usually adhered to the surface although they formed clumps after several days of incubation. The cell cultures of IOZCAS-Spex-II-A were composed of mostly spindle-shaped and a lot of spherical cells as depicted in Fig. 1*A*, whereas the parent Spex-II cell line consisted most of spheroid cells (Fig. 1*B*; Zhang et al. 2006a). The cloned round cells (mean 9.9±1.7 μm, $\bar{x}$±standard deviation) were obviously smaller than the parental cells (mean 14.7± 2.7 μm) of 30 duplicates respectively. Growth studies were performed on stationary cultures in 5 mL of growth medium in T-12.5 cm^2 glass flasks as described by McIntosh et al. (2001). The doubling time of Spex-II-A clone (28 h; Fig. 2) was 81 h shorter than that of Spex-II cell line (Zhang et al. 2006a). IOZCAS-Spex-II-A cell strain was confirmed as having originated from IOZCAS-Spex-II by DNA amplification fingerprinting technique employing polymerase chain reaction (DAF-PCR) analysis using aldolase as a primer. The primers (aldolase) set 1 (5′ CCG GAG CAG AAG AAG GAG CT) and set 2 (5′CAC ATA CTG GCA GCG CTT CA) and PCR reaction conditions used were modified from McIntosh et al.

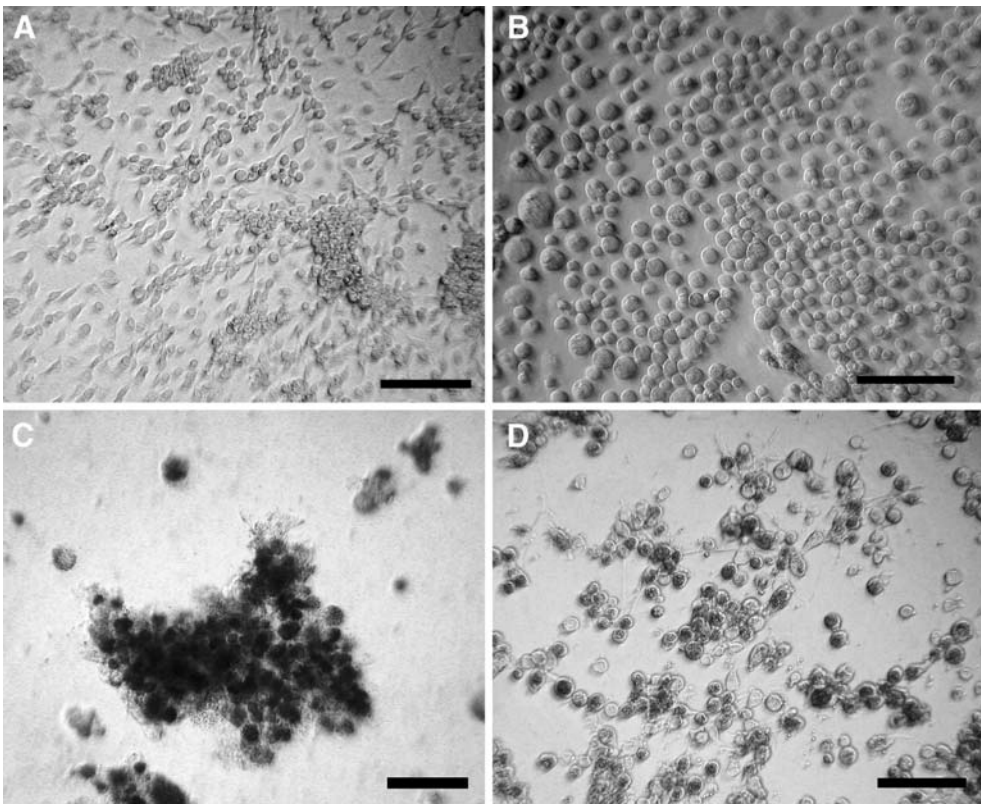

Figure 1. (*A*) Photomicrograph of IOZCAS-Spex-II-A showing predominantly spindle-shaped cells. *Marker bar* is 100 μm. (*B*) The parent Spex-II cell line consisted most of spheroid cells. *Marker bar* is 100 μm. (*C*) Cytopathology of the new cell clone (IOZCAS-Spex-II-A) infected with *S. exigua* NPV. Cells were infected and formed clumps which contained OBs. *Bar* 100 μm. (*D*) Cytopathology of the new cell clone (IOZCAS-Spex-II-A) infected with *A. californica* MNPV showing presence of OBs. *Bar* 100 μm.

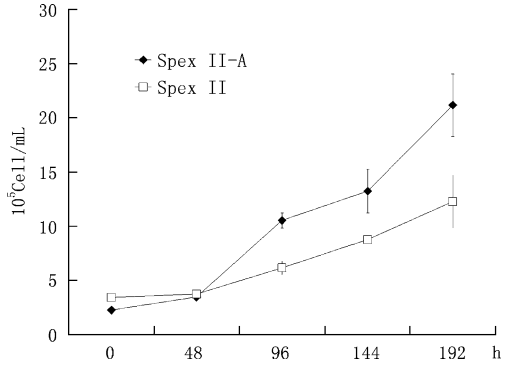

Figure 2. The growth curves of the new cell clone IOZCAS-Spex-II-A and its parent cell line IOZCAS-Spex-II.

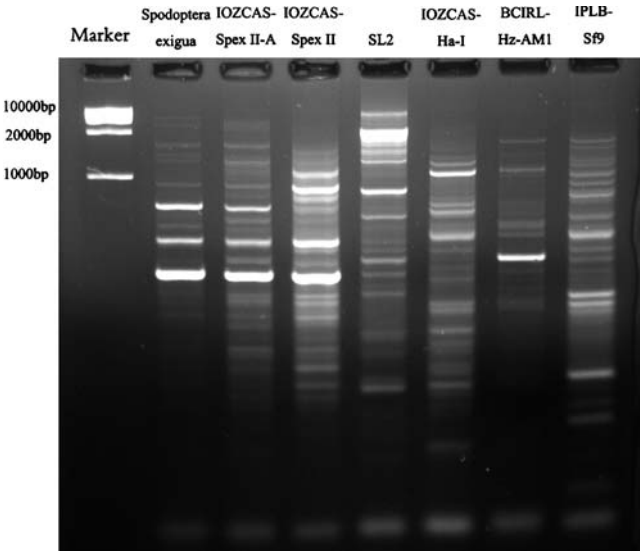

Figure 3. DAF-PCR profiles of the new cell clone and its parental cell line. The insect *S. exigua* and cell lines SL2, IOZCAS-Ha-I, BCIRL-Hz-AM1, and Sf9 served as comparisons.

(1996) and Liu et al. (2003). *S. exigua*, IOZCAS-Spex II-A, and IOZCAS-Spex-II results indicated three bands that were less than 1,000 bp. Additionally, the new cell clone had patterns that were distinct from all other cell lines maintained in this laboratory (Schneider's line SL2, Schneider 1972; IOZCAS-Ha-I, Zhang et al. 2006b; BCIRL-Hz-AM1, McIntosh et al. 1981; Sf9, Smith et al. 1985) as shown in Fig. 3.

Log phase cells were seeded in T25-cm^2 tissue culture flasks with approximately 2×10^6 cells and incubated at 27°C for 12 h. Following cell attachment, the medium was removed and 1 mL of third-passage *S. exigua* NPV budded virus (BV) was added at a multiplicity of infection of 0.01 pfu/cell (*S. exigua* NPV) and 0.1 pfu/cell (*A. californica* MNPV), respectively. The cells were incubated at 28°C for 1 h, rocked at 15 min intervals, and fed 5 mL fresh medium after third washes with the TNM-FH medium. The titer of BV was measured by the method of plaque analysis. Virus growth curve for *S. exigua* NPV in IOZCAS-Spex-II-A cells indicated that infectious BV was present in the medium by 24 h pi as indicated by an increase in titer. The highest BV titer was noted at 96 h pi to be 3.7×10^4 pfu/mL as showed in Fig. 4.

At 5 d pi, more than 90% of 2×10^6 IOZCAS-Spex-II-A cells were infected (Fig. 1*C*) and formed clumps which contained OBs whereas the parent cell line IOZCAS-Spex-II only had 50% of the infection. The calculated number of OBs recovered for the daughter cell line and the parent cell line were 7.1×10^7 and 2.4×10^7 OBs/mL with four duplicates, respectively. The number of OBs per cell of the cloned strain (IOZCAS-Spex-II-A) was 176±13 OBs/cell while the parental cell line produced 211±11 OBs/cell.

We also tested their respective susceptibilities to *A. californica* MNPV. The cloned cell strain and the parental cell line are each susceptible to *A. californica* MNPV (Fig. 1*D*). The titer of BV grew into the highest by 96 h of 1.3×10^6 pfu/mL after the inoculation as showed in Fig. 4.

The cell line Sf9 was also tested at the same time. OB level of the clone cell strain was 6.4×10^6 OBs/mL, with the parental line generating level of 3.4×10^7 OBs/mL, while the cell line Sf9 generated 6.8×10^6 OBs/mL. There was no significant difference in the yield of OBs between IOZ CAS-Spex-II-A and Sf9.

This demonstrates that IOZCAS-Spex-II-A is a better cell line for virus infection and production of *S. exigua* NPV than that of IOZCAS-Spex-II. However, IOZCAS-Spex-II was more suitable for *A. californica* MNPV infection.

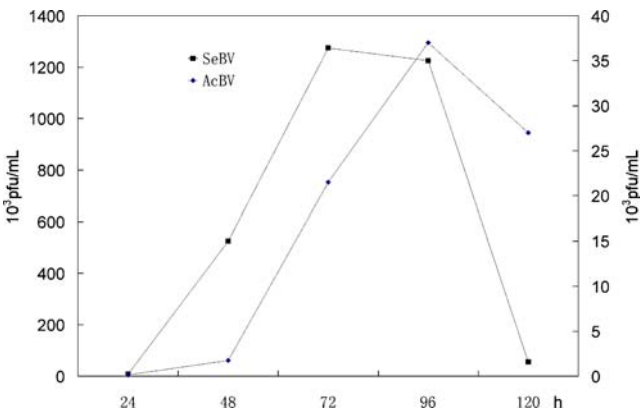

Figure 4. Virus growth curve for *S. exigua* NPV and *A. californica* MNPV in IOZCAS-Spex-II-A cells.

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