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REPORT

A new insect cell line from the pupal ovary of the Asian corn borer moth *Ostrinia furnacalis*

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Abstract Ovaries were removed from *Ostrinia furnacalis* (Guenée) pupae and were placed in a flask containing TNM-FH medium with 10% inactivated fetal bovine serum. Cell migration occurred after about 1 wk of the initiation in June 2011. The migrated cells were distributed over most of the flask and were treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), a chemical carcinogen, after about 1 mo of initiation for 26 d. Cells were first subcultured successfully 12 d after the MNNG was removed, followed by subculturing for 30 passages. The established cell line, designated IOZCAS-Osfu-1, were analyzed by DNA fingerprinting–PCR (DAF-PCR) to confirm that it originated from *O. furnacalis*.

Keywords *Ostrinia furnacalis* · Insect ovary cell line · MNNG

Carcinogenesis and the mechanisms of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) transformation have been extensively investigated through both in vitro and in vivo studies (Du et al. 1984; Su et al. 1995; Bunton and Wolfe 1996; Schar 2001; Izyumov and Talikina 2007). However, only a few studies have examined the use of MNNG to establish insect cell lines (Li et al. 2012). Cell lines from the larva fat body or embryos of *Ostrinia nubilalis* (Hübner) have been reported (Trisyono et al. 2000; Goodman et al. 2001); however, no published report is available for the cell lines from *Ostrinia furnacalis*, a major pest of corn in China (Wang et al. 2000). Establishment of the new insect cell lines is more valuable in the fields of baculovirus production and recombinant protein production in vitro and other biotechnology. This report presented the establishment and characterization of a new continuous cell line originated from the pupal ovaries of the insect.

Successful cultures were initiated in June 2011. Initiation and maintenance of the cell lines was as previously described (Li et al. 2012; Zhang et al. 2012). The female pupae were surface-sterilized by washing with 75% ethanol for 10 min. The ovaries were excised from the pupae, thoroughly rinsed with sterile Ringer's solution to remove hemocytes, and then rinsed with the primary medium. The medium employed to initiate primary cultures was TNM-FH (Sigma, Saint Louis, MO) containing 10% fetal bovine serum (FBS) and 10% TNM-FH saturated with phenylthiourea (primary medium). Whole tissues were subsequently transferred to a 25-cm^2 T-25 culture flask (Corning Incorporated, Corning, NY) containing 1.0 ml of primary medium. The cultures were incubated at 27°C. After an attachment period (ca. 12 h), an additional 3.0 ml of the growth medium was added without disturbing the attached tissues. The phenylthiourea-saturated TNM-FH solution was omitted from the growth medium. The migrated cells initially distributed themselves densely around tissue explants and then gradually moved to the surrounding areas and finally to the remainder of the flask after about 1 mo after initiation. Primary cultures were initially fed every 7 to 10 d (using 2-ml medium replacement).

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MNNG is a strong mutagen that causes chromosomal DNA damage by acting directly on nucleic acids. Therefore, MNNG successfully induced in vitro cell transformation in several cell lines of mammals (Gichner and Veleminsky 1982; Du et al. 1984; Milo et al. 1992; Malik et al. 1997; Ming et al. 2006). We used MNNG in our previous study to induce the division and transformation of cells cultured from the pupal ovary of *Spodoptera exigua* (Li et al. 2012). In the present study, an insect cell line derived from the pupal ovary of *O. furnacalis* was established by treating primary cultured cells with MNNG. When the cells approached confluence, they were treated with MNNG at a concentration of 5.0 μg/ml for 26 d. The first subculture was carried out 38 d after the beginning of MNNG treatment. The contents of the culture flask were then transferred to a new flask containing 2.0 ml of fresh growth medium. The interval of time between subcultures from the initial passage to the seventh passage ranged from 11 to 35 d, depending on the growth rate of the cells. After the seventh passage, the cells proliferated more rapidly, and thereafter, the interval of time between passages was 5 to 7 d using a ratio of cell suspension to fresh medium of 1:4 to 1:5. Sixty-eight cultures, with five pupal ovaries in each culture, were initiated simultaneously and 49 of these produced viable cells. Thirty-five of the latter cultures were incubated with 5 μg/ml MNNG, but only two of them grown into cell lines. We found that MNNG treatment overcomes previously described limitations to obtaining continually proliferating insect cells. Up to now, two of the cell line, designated IOZCAS-Osfu-1 and IOZCAS-Osfu-2, have been subcultured for more than 30 generations (Fig. 1) and were frozen at −105°C and successfully regenerated. Here, only the character of IOZCAS-Osfu-1 was described.

The morphology of the different cell types in the IOZCAS-Osfu-1 line was observed under phase contrast microscopy. The cells were heterogeneous in shape, consisting of spherical, spindle-shaped, and macrophage-like cells. The spherical cells were predominant among the various cell types

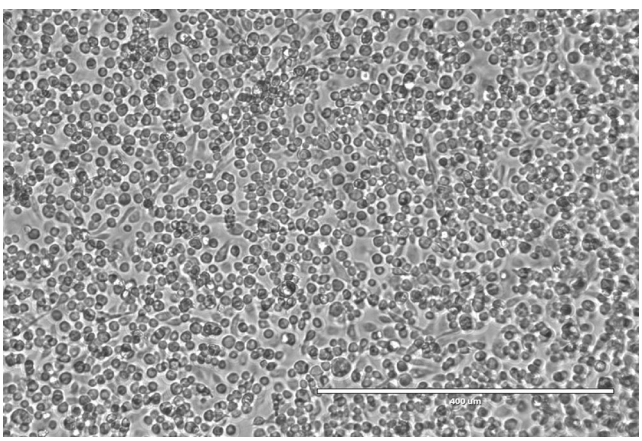

Figure 1. Cell monolayer of IOZCAS-Osfu-1 at passage 15. The *scale bar* is 400 μm.

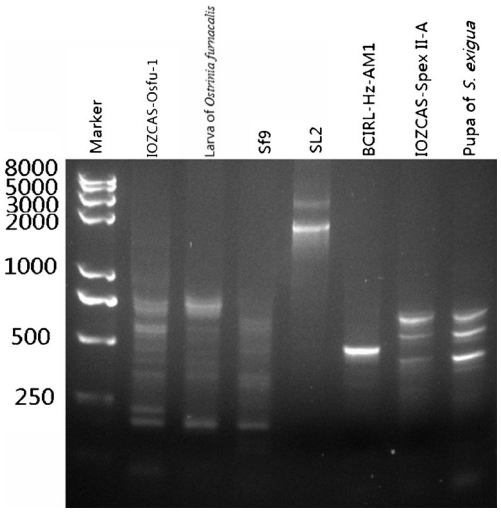

Figure 2. DAF-PCR profiles of IOZCAS-Osfu-1 cell lines and other cell lines in our laboratory, including Sf9, Sl2, BCRIL-HzAM1, and IOZCAS-Spex II-A.

(Fig. 1). The cell line was confirmed to have originated from *O. furnacalis* by utilizing the DNA fingerprinting–PCR (DAF-PCR) method and comparing the band profiles of the cell line with its host, using the aldolase sequence as a primer (McIntosh et al. 1996; Liu et al. 2003). The PCR reaction conditions used in this study have been previously described (Zhang et al. 2006). Similar profiles were seen between the new *O. furnacalis* cell line IOZCAS-Osfu-1 and its homologous host (*O. furnacalis* larvae). These profiles were distinctly different from those of other cell lines maintained in the laboratory (Fig. 2, Sf9, a cell line from *Spodoptera frugiperda*, Vaughn et al. 1977; SL2, a cell line from *Drosophila melanogaster*, Schneider 1972; BCIRL-Hz-AM1, a cell lines from *Heliothis zea*, McIntosh and Ignoffo 1983; IOZCAS-Spex II-A, a cell clone from *S. exigua*, Zhang et al. 2009).

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