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Research Article

Generation and characterization of a novel ovariole cell line derived from *Spodoptera frugiperda* in China with sensitivity to both SfMNPV and AcMNPV

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ABSTRACT

Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV), belonging to the species *Alphabaculovirus spofrugiperdae*, has been recently registered as an insecticide in China. This virus has a specific effect on the global major agricultural pest *Spodoptera frugiperda*. To gain insights into viral infection, replication processes, and the complex formation of viral particles, *in vitro* studies using cell lines are essential tools. Although the IPLB-Sf9 and IPLB-Sf21 cell lines derived from *S. frugiperda* are widely used for studies on the infection and replication mechanisms of Autographa californica multiple nucleopolyhedrovirus (AcMNPV), their capacity to produce viral polyhedra after SfMNPV infection is not optimal. To address this limitation, a novel cell line named IOZCAS-Sf-1 was developed from a *S. frugiperda* population in Yunnan, China. The mitochondrial *COX1* gene analysis confirmed the species origin of the IOZCAS-Sf-1 cell line. Furthermore, a comparative study was carried out to contrast the *COX1* gene sequence of this novel cell line with that of IPLB-Sf9, highlighting the distinctions between the two. Importantly, the IOZCAS-Sf-1 cells exhibited a remarkable ability to generate polyhedra when infected with AcMNPV and SfMNPV, respectively. Consequently, this cellular lineage is considered a promising and valuable resource. It serves not only to investigate the molecular mechanisms of viral replication and its impact on host cells, but also to explore the transfection efficiency of SfMNPV DNA. This exploration further expands into its potential application in recombinant DNA experiments, laying a theoretical groundwork for the advancement of more effective biopesticides and sustainable agricultural practices.

1. Introduction

Spodoptera frugiperda (Lepidoptera: Noctuidae) poses a substantial threat to agriculture by feeding on more than 350 plant species, including major crops such as corn, rice, cotton, soybeans, and sorghum (Casmuz et al., 2010). Originally native to tropical and subtropical regions of the Americas, it was first introduced to Africa in 2016 (Jeger et al., 2017), reaching the Chinese mainland in 2019 and quickly spreading to form distinct geographical populations (Cheng et al., 2022). Owing to its robust reproductive and migratory capabilities, coupled with its polyphagous nature and short life cycle without diapause, the control of *S. frugiperda* necessitates advanced pest management technologies. The evolution of resistance in *S. frugiperda* to traditional chemical pesticides has underscored the need for alternative approaches (Blanco et al., 2010; León-García et al., 2012), with biological pesticides offering a promising solution.

Insect viruses, particularly baculoviruses, are specific to pests, causing minimal harm to the environment and nontarget organisms. Moreover, the use of biopesticides can result in persistent and natural control of pest populations, potentially leading to epidemics that endure across generations (Mei et al., 2020). The primary focus of viral microorganism-based control for *S. frugiperda* relies on Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) (Bateman et al., 2021), a member of the species *Alphabaculovirus spofrugiperdae*, which has a narrow host range limited to *S. frugiperda*, *S. litura* and *S. exigua* (Adams and McClintock, 1991; Hamm and Styer, 1985). It can manage the pest population by reproducing and dispersing within *S. frugiperda*, resulting in the eventual demise of the host larva.

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Although numerous SfMNPV strains have been successfully isolated globally (Arive, 2014; Barrera et al., 2011, 2015; Berretta et al., 1998; Escribano et al., 1999; Masson et al., 2021; Simón et al., 2011, 2012; Vieira et al., 2012; Wolff et al., 2008), significant variations exist in their virulence, including their lethal concentrations and rates. Genetic variation among isolates contributes to their differences in virulence (Bateman et al., 2021; Simón et al., 2011, 2012; Wolff et al., 2008). Therefore, investigating the biological characteristics, infection mechanism, and interaction between SfMNPVs and host cells is essential for advancing the development of highly efficient biopesticides. Various populations of *S. frugiperda* exhibit diverse responses to the same SfMNPV strain. Bioassay experiments have shown different susceptibilities of larvae from corn and rice strains to identical SfMNPV isolates, with corn strains exhibiting a broader spectrum of susceptibility than rice strains in terms of both lethal concentration and killing speed (Popham et al., 2021). Geographical populations of *S. frugiperda* in China also show variations in sensitivity to SfMNPV (Cheng et al., 2022). Therefore, it is essential to acquire indigenous SfMNPV isolates to effectively control local populations of *S. frugiperda* (Hussain et al., 2021).

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is a member of the species *Alphabaculovirus aucalifornicae* within the genus *Alphabaculovirus,* family *Baculoviridae.* AcMNPV has a relatively broad host range and can infect the larvae of at least 32 lepidopteran species, including *S. frugiperda* (Granados and Williams, 1986). However, *S. frugiperda* is considered a semipermissive host for AcMNPV, with its larvae exhibiting high resistance to oral infection by AcMNPV, which presents challenges for effective field control. This resistance stems from the inability of occlusion-derived virus (ODV) from AcMNPV to establish primary infection in the midgut cells of *S. frugiperda*, rendering oral infection ineffective (Haas-Stapleton et al., 2003). Nevertheless, *S. frugiperda* shows high susceptibility to AcMNPV budded virus (BV) infection initiated in the hemocoel, with a median lethal concentration (LD_{50}) of less than 1 p.f.u. (Haas-Stapleton et al., 2003).

The purification of different genotype strains could be carry out *in vivo* (passage in live larvae) (Lei et al., 2020) or *in vitro* (in insect cell lines). In particular, *in vitro* plaque purification techniques using insect cell lines have facilitated the isolation of individual genotypic viral strains. This approach proves to be an effective method for the isolation of naturally occurring variants from field isolates, with the potential to possess more desirable characteristics. The commonly used cell line IPLB-Sf21 and its derivative IPLB-Sf9 are derived from the ovaries of *S. frugiperda* and play crucial roles in insect virology research (Vaughn et al., 1977), especially for AcMNPV (Goodwin et al., 1970; Jarman--Smith et al., 2002). Initial reports indicated that IPLB-Sf21 cells could support replication process of SfMNPV (Goodwin et al., 1973). Later studies showed that IPLB-Sf9 cells could form plaques after being inoculated with ODV (Clavijo et al., 2010). However, in our observation, only a few IPLB-Sf9 cells will produce polyhedra after inoculation with BV of SfMNPV, which may become an obstacle to further explore the interaction mechanism between virus and host cells. In order to solve this problem and further understand the infection process, pathogenic mechanism and develop effective antiviral strategies, it is essential to develop additional SfMNPV-sensitive cell lines. Furthermore, it is important to note that several cell lines originating from *S. frugiperda*, such as IPLB-Sf9, are able to be infected with Sf-rhabdovirus. Virus-infected cell lines can transmit the virus vertically to their derived cell lines. Within the baculovirus expression vector system (BEVS), the presence of rhabdovirus may interfere with the efficiency and quality of baculovirus target protein expression by competitively occupying host cell resources or affecting the replication and transcription process of baculovirus. Therefore, it is necessary to obtain a rhabdovirus-free Sf cell line (Maghodia et al., 2016).

Although there are 111 records of cell lines derived from *S. frugiperda* in the Expasy database (https://web.expasy.org/cellosaurus/), there are only 30 records of original newly established cell lines after excluding the cell lines derived from IPLB-Sf21 (Supplementary Table S1). These other cell lines mainly originate from various larval tissues, including the imaginal disc (Lynn and Oberlander, 1983), nervous system, aorta, testes (Reall et al., 2019), midgut (Zhou et al., 2020) and fat body (Wang et al., 2021). In addition to IPLB-Sf21, only one other cell line, IPLB–SF–1254, which was reported simultaneously with IPLB-Sf21, originates from the ovaries of pupae (Vaughn et al., 1977).

In this study, we have successfully established a *S. frugiperda*-derived cell line that facilitates the *in vitro* replication and proliferation of SfMNPV. The new cell line, named IOZCAS-Sf-1, was identified on the basis of the mitochondrial cytochrome *c* oxidase subunit I (*COX1*) gene and differs from the IPLB-Sf9 cell line. Upon infection with AcMNPV and SfMNPV, the IOZCAS-Sf-1 cell line exhibited the ability to produce polyhedra.

2. Materials and methods

2.1. Primary culture and subculture

S. frugiperda, acquired from Yunnan Province, China, was subsequently reared on artificial diets at 25 °C in the laboratory at the Institute of Zoology, Chinese Academy of Sciences in Beijing, China.

The primary culture medium consisted of a mixture of 10% fetal bovine serum (FBS) and 90% Insect-XPRESS™ (12-730Q, BioWhittaker, Lonza, Walkersville, MD, USA). This medium was further supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL). Additionally, the primary culture medium had a specific ratio of 8:1:1 (Insect-XPRESS™ medium: FBS: Insect-XPRESS™ medium saturated with phenylthiourea solution).

The primary culture and subculture procedures were adapted from Tong's methodology (Tong et al., 2021), which was developed for the ovarian cell line IOZCAS-Myse-1. In summary, pupae in the middle stage of development were collected, and the ovarioles were extracted after disinfection and then placed in a culture flask. A critical step was to secure the ovariole to the flask bottom to facilitate cell release. The primary culture medium was subsequently added gradually. After 21 days of primary culture, the cells were subjected to a 9 days of treatment with 5% hypoxia, after which the medium was replaced to restore the normoxic conditions. The first subculture was conducted approximately one month after restoring normoxic conditions. Depending on the cell growth rate, the time interval between each subculture ranged from 1 to 3 weeks, from the initial passage to the 10th passage. After the 10th passage, the subculture intervals were shortened. After each subculture, 1/2 to 4/5 of the volume medium was replaced. The newly established cell line was designated IOZCAS-Sf-1 (IOZCAS, referring to the Institute of Zoology, Chinese Academy of Sciences).

2.2. Species origin identification of DNA barcoding based on COX1 gene sequences

DNA was isolated from 20th passage of IOZCAS-Sf-1, IPLB-Sf9, and *S. frugiperda* larval hemocytes. Cells of the cell line were collected by centrifugation. Anatomical scissors were used to cut a foot off of *S. frugiperda* larva and collect the hemocytes that flowed out. PrimeSTAR® Max DNA Polymerase (Takara, Cat. No: R45Q) was used for PCR amplification. Primers (SfCOX1F and SfCOX1R Supplementary Table S2) were designed for the partial CDS sequence of cytochrome *c* oxidase subunit I gene (*COX1*, Gene ID: 25768279) according to the complete *S. frugiperda* mitochondrial genome (GenBank accession number: NC_027836.1), with an expected product size of 811 bp. Each PCR had a total volume of 50 μL, comprising PrimeSTAR Max (2 ×) 25 μL, 20 pM primers, and 70 ng of the cell DNA template. PCRs were carried out at 94 °C for 5 min for initial denaturation, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, with a final extension at 72 °C for 10 min. Subsequently, PCR products were separated via 1% agarose gel, ligated into the pEASY-T1 vector (Beijing TransGen Biotech Co., Ltd.), and sequenced directly by Sangon Biotech Co., Ltd., Shanghai, China.

The *COX1* gene partial sequences obtained from IOZCAS-Sf-1, IPLB-Sf9, and hemocytes of *S. frugiperda* from the Yunnan population in China were submitted to NCBI (GenBank accession numbers: PP727358, PP727359, PP727360) and ScienceDB (Accession link: https://www.scidb.cn/anonymous/N1paN2Jp). Sequence analysis was conducted using the DNASTAR software package (DNASTAR, Madison, WI, USA). After the vector sequence was removed, sequence alignment was carried out by Clustal W using MegAlign software.

2.3. Morphological observation and size measurement of cells

Images of cells derived from both the primary culture and the established cell line were captured using a Leica inverted phase contrast microscope (Leica, Germany, DM2000) equipped with a charge-coupled device (CCD) camera system (TOUPCAMTM, Suzhou, China, E3CMOS). The cell size was calculated on the basis of a calibrated magnification factor. The average cell dimensions were determined through the measurement of 90 cells. Statistical analysis was conducted with GraphPad Prism 8.0.2 software. The differences between generations and cell lines were assessed using analysis of variance (ANOVA) and *t*-tests. All of the statistical tests were conducted at a confidence level of 95%.

2.4. Population growth of IOZCAS-Sf-1 cells

The cells in the logarithmic growth phase (passage 20) were seeded into T-25 cm^2 culture flasks at a concentration of 2.0×10^5 cells/mL. Each flask contained 5 mL of growth medium, and the culture process was conducted at 27 °C for a duration of 6 days, with four replications. The cells were subsequently counted via a hemocytometer, and the cell population doubling time was subsequently determined using the exponential formula outlined by Kuchler (1977).

2.5. Flow cytometry

Flow cytometry was used to detect chromosome multiples, with hemocytes from *S. frugiperda* larvae serving as the diploid control. The cells were collected at a density of approximately 1×10^6 cells/mL and fixed overnight at 4 °C in 70% ethanol. Subsequently, they were treated with 0.1 mg/mL propidium iodide (PI) for 30 min at 37 °C. Detection was conducted using flow cytometry (BD Biosciences, East Rutherford, NJ, USA, LSR Fortessa) after filtration.

2.6. Baculovirus infection

Cells in the logarithmic growth phase were seeded in T-25 cm^2 culture flasks at a density of 2×10^6 cells per flask. The cells were subsequently cultured in growth medium at 27 °C for 12 h to allow them to adhere to the bottom of the flask. The BVs of wild-type AcMNPV strain, preserved in our laboratory (Jin et al., 2021), were used to infect cells at a multiplicity of infection (MOI) of 0.1. SfMNPV BVs were obtained from the hemolymph supernatant of SfMNPV-susceptible *S. frugiperda* larvae previously infected with SfMNPV (KYc01). Briefly, the hemolymph supernatant was filtered at 0.22 μm, diluted 100 times, and used to infect cells. The culture flasks were gently shaken for 3 h on a vertical shaker to facilitate viral adsorption. After 6 days, the cells were observed under an inverted phase contrast microscope and photographed. The number of polyhedra formed within the cells was used as the index to calculate the infection rate.

2.7. Virus proliferation curve

The virus proliferation curve was generated via a plaque assay. The cells were seeded at a density of 1×10^6 cells/well in a 6-well plate and allowed to adhere for 2 h. AcMNPV and SfMNPV were separately used to infect cells at a MOI of 0.1. The culture supernatant was collected from each well daily for 6 days.

For the plaque assay, cells were inoculated into a 24-well plate at a density of 4×10^5 cells/well. After the cells adhered to the bottom, the supernatant was removed, and the viral supernatant was added at gradient dilutions ranging from 10^{-2} to 10^{-7}. After infection for 2 h, the viral supernatant was discarded, and a 0.75% agarose solution dissolved in the medium was added. After 6 days, plaque counts were determined via microscopy, the virus titer was calculated, and the virus proliferation curve was plotted.

2.8. Statistical analysis

Statistical analysis was performed and graphs were created using GraphPad prism 8.0.2. The differences among all samples were assessed

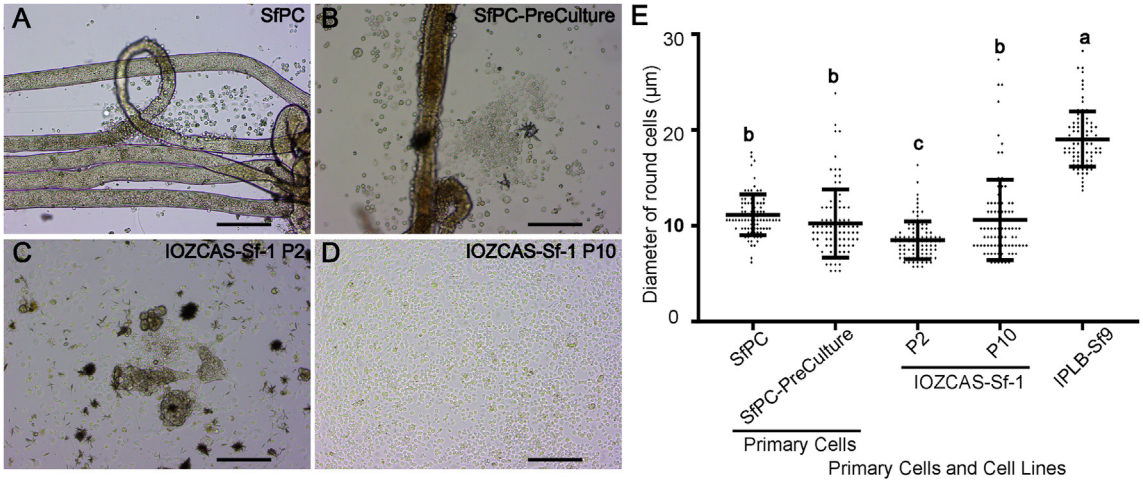

Fig. 1. The isolation and culture of primary cells from the ovarioles of middle-stage *Spodoptera frugiperda* pupae. **A** Migration process of primary cells within 48 h after explanting. The ovarian lumen cells were located between the outer epidermis of the long tubular ovariole (tunica extema) and the internal ovariole tissue (tunica propria). **B** The state of cells in the ovariole of explants, along with their release behavior, observed under an optical microscope on the 60th day of *in vitro* cultivation. **C** Cell clusters of proliferating cells 14 days after the 2nd passage. **D** The 10th generation of IOZCAS-Sf-1 cells. Scale bar = 100 μm in **A, B, C** and **D**. **E** Variability in the diameters of primary cells and subcultured IOZCAS-Sf-1 cells at the 2nd passage (P2) and the 10th passage (P10), and of IPLB-Sf-9 cells. The same letter indicate no significant difference in the cell diameters. The data are presented as mean ± standard deviation (SD) of relative expression level. The differences between generations and cell lines were assessed using analysis of variance (ANOVA) and *t*-tests.

using analysis of variance (ANOVA) and *t*-tests. All tests were performed with a confidence limits at 95%.

3. Results

3.1. Primary cultures and subcultures

The culture flask was equipped with five sets of ovaries from female pupae. Cell migration occurred within 48 h after planting (Fig. 1A). Initially, the cells densely surrounded the explants but gradually migrated to adjacent areas, eventually covering almost the entire surface of the culture flask. On the 60th day after *in vitro* culture, the cells in the ovariole of the explant were close to apoptotic, but the cells released from the explant still survived and proliferated into cell clusters (Fig. 1B). After 9 days of hypoxia induction and 21 days of reoxygenation, numerous cell clusters became visible under the microscope (Fig. 1C). The cells subsequently underwent their first subculture, and the subcultured cells remained viable. Initially, the proliferation rate during early passages was slow, requiring prolonged subculturing times. However, subculturing intervals accelerated, and stabilized by the 10th passage (Fig. 1D), subculturing was carried out at a ratio of 1:5 of cell suspension to fresh culture medium.

Initially, the primary cells exhibited a uniform morphology (Fig. 1A) but increased in diversity after subsequent proliferation. Notably, the cells displayed considerable variation in size over the first 10 passages (Fig. 1E). Among the different cell types in IOZCAS-Sf-1, spherical cells were predominant, with an average diameter of 10.62 ± 4.18 μm (Fig. 1E). Furthermore, compared with IPLB-Sf9 cells (19.03 ± 2.85 μm),

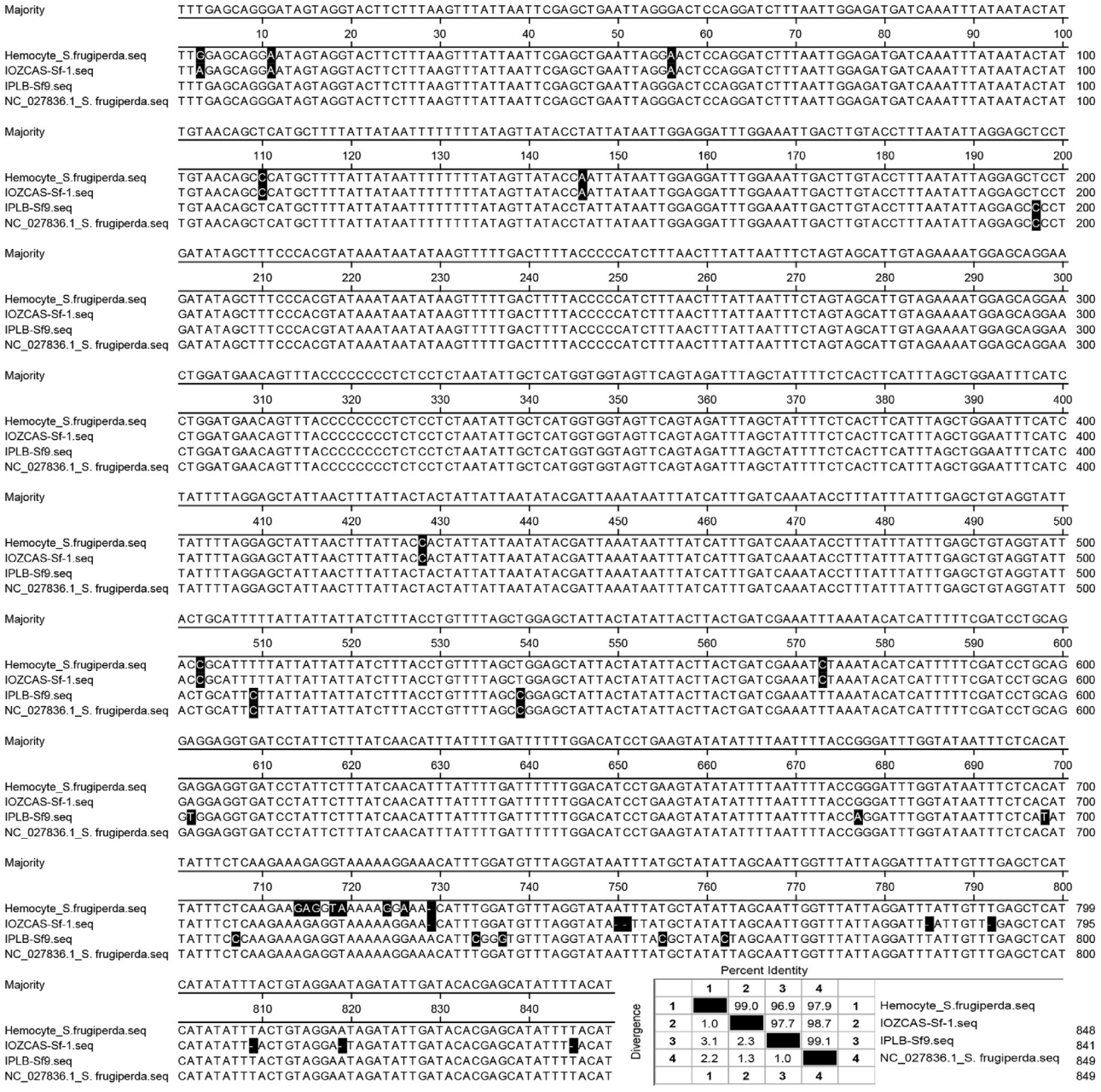

Fig. 2. IOZCAS-Sf-1 was distinguished from IPLB-Sf9. The mitochondrial *COX1* gene sequences alignment was performed using Clustal W with MegAlign software. The *Spodoptera frugiperda* hemocyte DNA was obtained from *Spodoptera frugiperda* found in Yunnan, China. The mitochondrial DNA sequences of IOZCAS-Sf-1 and IPLB-Sf9 cells were acquired through sequencing. The *S. frugiperda* data are obtained from the NCBI Genebank (Accession number: NC_027836.1), which contains a complete mitochondrial DNA sequence.

IOZCAS-Sf-1 cells were notably smaller (Fig. 1E), rendering them resistant to greater shear forces and facilitating large-scale culture and production.

3.2. IOZCAS-Sf-1 is homologous to S. frugiperda

The mitochondrial *COX1* gene serves as a coding region within mitochondrial DNA, exhibiting variability among different species. By comparing and analyzing *COX1* gene sequences, both population genetics and phylogenetic relationships can be explored. The *COX1* analysis method is commonly employed to determine relationships between species or subspecies, as well as to identify unknown biological samples. By comparing with the *COX1* gene with *COX1* sequences of known species, the species classification and origins of cell lines can be confirmed, thereby verifying the species origin of specific cell lines. To verify the species origin of IOZCAS-Sf-1, we obtained a partial nucleotide sequence of the *COX1* gene from our IOZCAS-Sf-1 cell line, the IPLB-Sf9 cell line and hemocytes from a local Yunnan strain of *S. frugiperda* through PCR and Sanger sequencing (Fig. 2). Analysis revealed that the *COX1* gene sequence of IPLB-Sf9 shared a 99.1% match with the NCBI sequence NC_027836.1 (Fig. 2). The 848 bp sequence of the *COX1* gene from the hemocyte cells of *S. frugiperda* in Yunnan, China, exhibited a 99.0% match with an 841 bp sequence of IOZCAS-Sf-1, and a 97.9% match with the NCBI sequence NC_027836.1 (Fig. 2). Notably, further differences were observed in the *COX1* gene sequences between IOZCAS-Sf-1 and IPLB-Sf9, with 97.7% identity (lower right corner of Fig. 2). These findings strongly support the conclusion that IOZCAS-Sf-1 originated from the *S. frugiperda* population in China and is distinct from IPLB-Sf9.

3.3. Characteristics of IOZCAS-Sf-1

The cell growth curve was determined and analyzed via the cell population doubling time formula (Kuchler, 1977). The result revealed that the population doubling time of IOZCAS-Sf-1 was 46.98 h, whereas that of IPLB-Sf9 was 46.01 h (Fig. 3A).

Counting the numerous small chromosomes of Lepidoptera can be quite challenging. Flow cytometry provides a method to correlate the number of cell events with the integral signal of PI fluorescence. In this study, we utilized larval hemocyte cells from *S. frugiperda* as a diploid control (Fig. 3C). Using flow cytometry to analyze the cell cycle, the cells were categorized into different phases on the basis of their DNA content: G0/G1 phase (diploid), S phase (2–4 tetraploid), and G2/M phase (tetraploid). Hemocytes initially exhibited a fluorescence intensity peak at approximately 25 K at 360 V (Fig. 3C), whereas IOZCAS-Sf-1 presented a first fluorescence intensity peak at approximately 100K (Fig. 3D), indicating that it is four times greater than that of the diploid control and

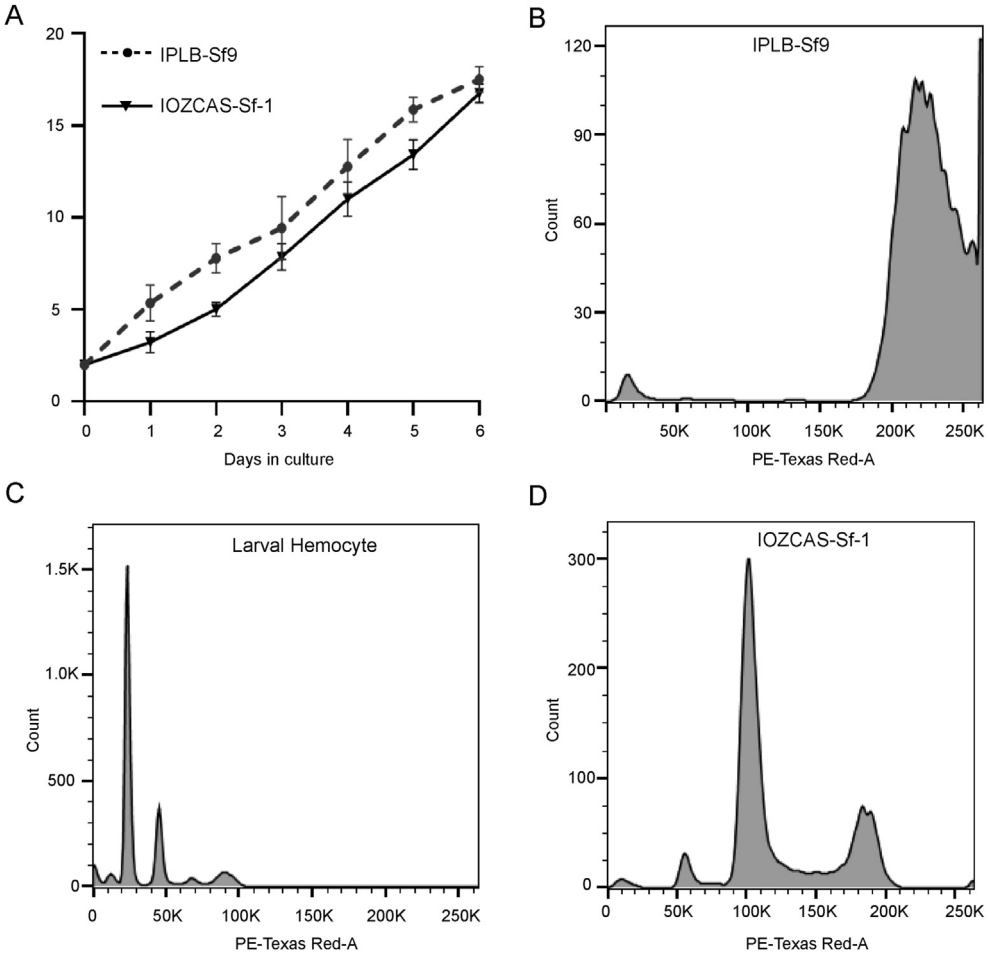

Fig. 3. Cell biological characteristics of IOZCAS-Sf-1. **A** The growth curve of the 20th generation of IOZCAS-Sf-1. The cells were counted by a hemocytometer at various time points. The cell population doubling time was calculated by the exponential formula described by Kuchler (1977) and the data are reported as mean ± standard deviation (SD). **B-D,** Flow cytometry was used to detect chromosome multiples. The cells were treated with propidium iodide (PI) after collected, fixed and filtrated. The cells were categorized into different phases on the basis of their DNA content: G0/G1 phase (diploid), S phase (2–4 tetraploid), and G2/M phase (tetraploid). **B** IPLB-Sf9 has a peak value of 200K. **C** Hemocytes of *Spodoptera frugiperda* larvae serving as the diploid control which exhibit an initial fluorescence intensity peak at approximately 25 K at 360 V. **D** IOZCAS-Sf-1 displays a first fluorescence intensity peak at approximately 100 K.

suggesting that IOZCAS-Sf-1 is octoploid. In contrast, IPLB-Sf9 had a peak value of 200K (Fig. 3B), indicating that it is closer to 16 ploidy than the other cell types are.

3.4. IOZCAS-Sf-1 cells support AcMNPV and SfMNPV replication

IOZCAS-Sf-1 cells displayed susceptibility to AcMNPV, characterized by cytopathogenic features such as bulging cells and enlarged nuclei containing numerous occlusion bodies (OBs) during the late stages of infection, observed as polyhedral particles in Fig. 4A. The AcMNPV infection rate in IOZCAS-Sf-1 cells reached 49.81% on day 6 post infection (Fig. 5A). Employing the same testing methodology, IOZCAS-Sf-1 cells were also sensitive to SfMNPV (Fig. 4B), with an infection rate of 36.32 % on day 6 post infection (Fig. 5A). In parallel studies, IPLB-Sf9 cells were used as a control and exhibited high sensitivity to AcMNPV infection (Figs. 4C and 5A), whereas the SfMNPV infection rate in IPLB-Sf9 cells was only 1.11 % (Figs. 4D and 5A). Furthermore, when the same cell concentration was used to inoculate AcMNPV, the yield of polyhedra produced by IOZCAS-Sf-1 cells was 0.73×10^6 OBs, whereas in IPLB-Sf9 cells the yield was 1.49×10^6 OBs. However, when SfMNPV was inoculated, the polyhedra yield in IOZCAS-Sf-1 cells was 20 times greater than that in IPLB-Sf9 cells (Fig. 5B). Regarding the dynamics of viral infection, the virus titer was initially low after infection with AcMNPV in both IOZCAS-Sf-1 and IPLB-Sf9 cells but increased rapidly on day 3 and stabilized in the later stage. The final virus titer in IOZCAS-Sf-1 cells was significantly lower than that in IPLB-Sf9 cells (Fig. 5C). Following infection with SfMNPV, the virus titer in IOZCAS-Sf-1 cells continued to increase within 6 days, whereas that in IPLB-Sf9 cells remained relatively limited (Fig. 5D).

4. Discussion

There is consistently a steady demand for insect cell lines that are sensitive to specific viruses, and SfMNPV fits well into this requirement. SfMNPV replicates exclusively in *S. frugiperda* cells (McIntosh et al., 1985). IPLB-Sf21 is derived from *S. frugiperda*, and IPLB-Sf9 is a clone of IPLB-Sf21 cells. IPLB-Sf21 cells can support the replication of SfMNPV (Goodwin et al., 1973; Knudson and Tinsley, 1974). The IOZCAS-Sf-1 cell line reported in this study only the second ovarian cell line derived from *S. frugiperda* since 1977 (Vaughn et al., 1977).

Knudson and Tinsley (1974) revealed the relationship between plaque analysis and terminal dilution analysis of SfNPV-2 in the IPIB-SF-21AE cell line (Crawford and Sheehan, 1983). However, when the mean tissue culture infectious dose ($TCID_{50}$) of IPLB-Sf21 cells infected with AcMNPV (1.8×10^8/mL) is compared with that of those infected with SfMNPV (1×10^6/mL), there is a significant 180-fold difference between the two values (McIntosh et al., 1985). Furthermore, Danyluk and Maruniak (1987) reported that the $TCID_{50}$ of AcMNPV in IPLB-Sf21 cells ($10^{7.6}$) was significantly greater than that in cells infected with SfMNPV ($10^{4.9}$), with a difference of 501 times. In our research facility, we observed abundant production of polyhedra in IPLB-Sf9 cells infected with AcMNPV. However, when SfMNPV is introduced, the yield of polyhedra decreases to only about 1%. This difference in production could be attributed to the altered characteristics of IPLB-Sf9 cells resulting from multiple passages in our laboratory. Consequently, the virus's infection and sensitivity might have been affected. To comprehensively elucidate the intricate interaction mechanism between SfMNPV and the host cell line IPLB-Sf9, additional investigations are imperative.

Early observations during the establishment of an ovarian cell line from *Mythimna separata* revealed that the primary cells originated from the lumen cells of the ovariole, which are somatic cells rather than germ cells or follicular epithelium cells (Tong et al., 2021). Considering that both *M. separata* and *S. frugiperda* belong to Lepidoptera and share similar developmental processes, insights from the establishment process suggest that the IOZCAS-Sf-1 cell line, which was established from the ovariole of the middle-stage pupa, likely also originates from lumen cells.

The small and numerous chromosomes in Lepidoptera pose challenges for accurate counting under an optical microscope. Previous studies have focused primarily on determining the chromosome count in Lepidoptera insects, suggesting an estimated chromosome count of approximately 30 in *S. frugiperda*. Although the exact number of

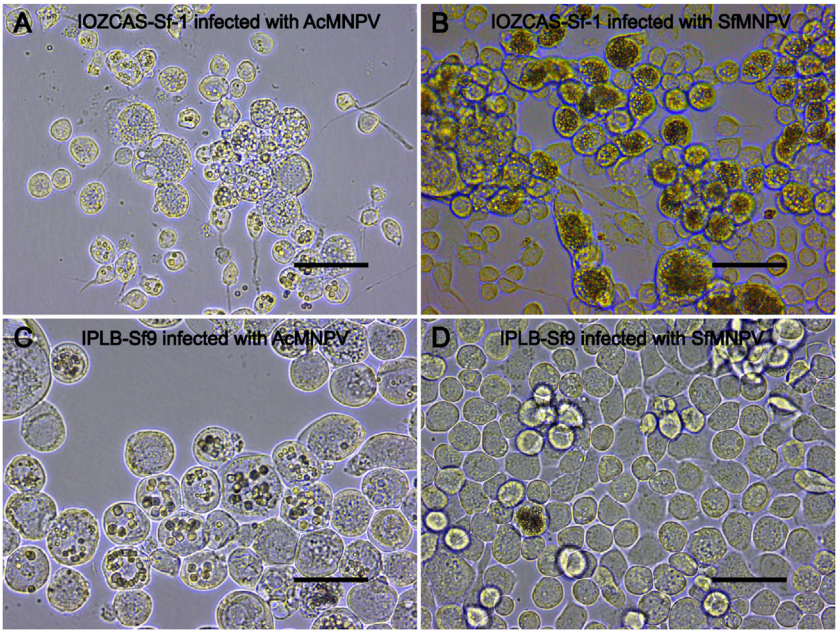

Fig. 4. AcMNPV and SfMNPV exposure induces polyhedral particle formation in IOZCAS-Sf-1 cells. Cells were inoculated in a cell culture flask and infected with viruses at an MOI = 0.1. The cell morphology and internal structure were observed at day 3 after infection. The polyhedron in the cell is the inclusion body produced by virus. Scale bar = 50 μm. **A** IOZCAS-Sf-1 at the 25th passage infected with AcMNPV. **B** IOZCAS-Sf-1 at the 25th passage infected with SfMNPV. **C** IPLB-Sf9 infected with AcMNPV. **D** IPLB-Sf9 infected with SfMNPV.

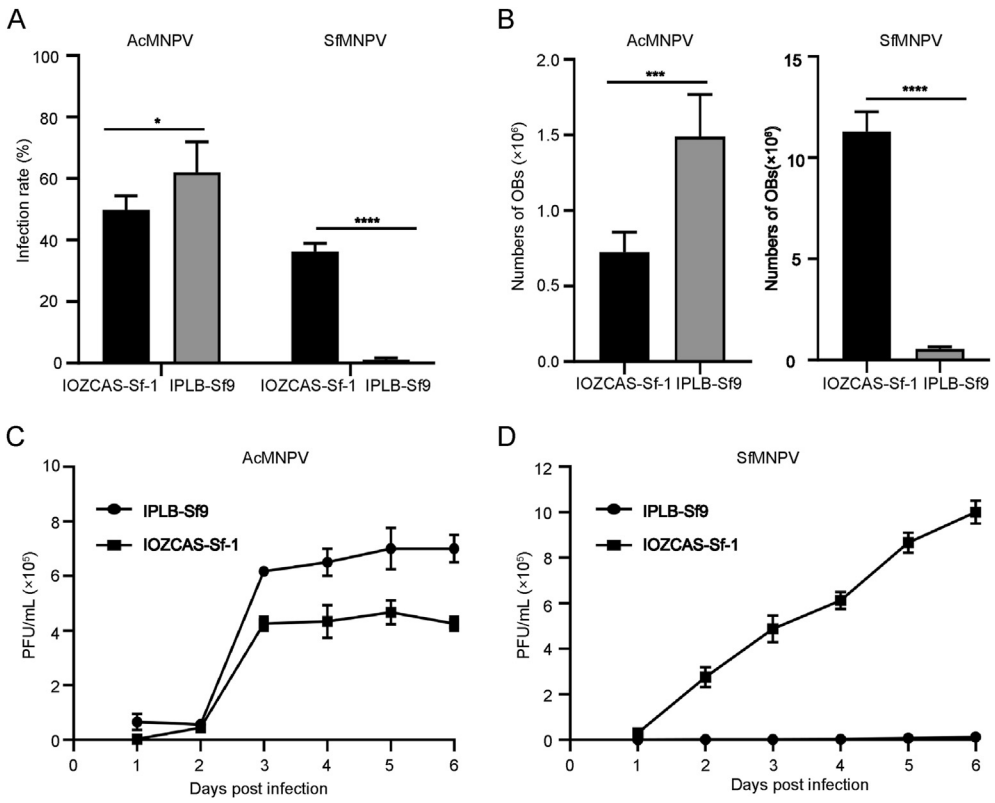

Fig. 5. Infection rate and viral production in IOZCAS-Sf-1 cells infected with AcMNPV and SfMNPV. **A** Statistics on the infection rates of AcMNPV and SfMNPV in the IOZCAS-Sf-1 cells at the 25th passage. The infection rate was calculated according to the infected and uninfected cells in Fig. 4. **B** Polyhedral yield of AcMNPV and SfMNPV in IOZCAS-Sf-1 cells. The cells were set in cell culture flask (2×10^6 cells per flask) and infected by AcMNPV or SfMNPV (MOI = 0.1). Six days after inoculation, the polyhedron concentration was harvested and counted with a hemocytometer. **C, D** Proliferation curve of AcMNPV (**C**) and SfMNPV (**D**) in IOZCAS-Sf-1 cells. IOZCAS-Sf-1 and IPLB-Sf9 cells were infected with AcMNPV and SfMNPV. The budded virus (BV) concentration was determined by plaque assay. The data are presented as mean ± standard deviation (SD) of relative expression level. The differences in virus sensitivity among different cell lines were statistically analyzed by ANOVA and *t*-test.

S. frugiperda chromosomes was not determined via genome sequencing and analysis of IPLB-Sf21 and IPLB-Sf9 (Kakumani et al., 2014; Nandakumar et al., 2017), subsequent studies conducted chromosome-level genome sequencing on *S. frugiperda*. These investigations confirmed that the species has a total of 31 chromosomes (Gimenez et al., 2020; Liu et al., 2019; Xiao et al., 2020; Zhang et al., 2019). This finding enhances our understanding of the chromosomal composition of *S. frugiperda* and offers valuable insights into the genetics of Lepidoptera.

Despite the widespread use of IPLB-Sf9 or IPLB-Sf21 cells as vectors in BEVS, the polyploid nature of IPLB-Sf9 cells has often been overlooked (Jarman-Smith et al., 2002, 2004). Previous cytogenetic studies revealed that IPLB-Sf9 cells possess approximately 130 chromosomes, indicating a highly polyploid or mixed ploidy state (Léry et al., 1999; Vaughn et al., 1977). In 2000, Doverskog et al. reported the emergence of an octoploid subgroup during G2/M stagnation, further highlighting the complex nature of IPLB-Sf9 cells (Doverskog et al., 2000). According to a study conducted by Jarman-Smith and colleagues in 2002, the IPLB-Sf9 cell line is a diverse combination of both “diploid” and “tetraploid” cells. Analysis of the chromosome number in the “diploid” cells revealed a range of 153 to 231 chromosomes, with an average of 198 (+33, −45) chromosomes. On the other hand, the “tetraploid” cells presented chromosome numbers ranging from 376 to 451, with an average of 426 (+25, −50) chromosomes (Jarman-Smith et al., 2002, 2004). In this study, we identified IOZCAS-Sf-1 as an octoploid through flow cytometry analysis of the cell cycle. To establish a point of reference, we utilized the larval blood cells of *S. frugiperda* as a diploid control. In contrast, IPLB-Sf9 is closer to 16 ploidies. Generally, polyploid cells have more chromosomes and organelles than do low-ploidy cells within the same cell line, enabling them to produce more proteins and metabolites, resulting in a larger cell size. However, high ploidy numbers can also lead to slower cell growth rates, as these cells require more time and energy to replicate their chromosomes, increasing the complexity of DNA replication and repair processes and increasing the risk of abnormalities or mutations. In cell culture, especially in large-scale culture systems, shear force is a factor that cannot be ignored, and shear resistance refers to the ability of cells to maintain their integrity and function when subjected to fluid shear forces. Smaller cells may adapt easily to different culture conditions and maintain their integrity and function when subjected to fluid shear forces (Chalmers, 1996; Weidner et al., 2017). Thus IOZCAS-Sf-1 cells might be more stable and controllable during large-scale culture and production, which is crucial for preventing cell damage and enhancing production efficiency.

Numerous *S. frugiperda* cell lines have been contaminated by rhabdovirus, and this contamination can be transmitted vertically to subsequent cell lines. In the BEVS, the presence of rhabdovirus may impact the competition for cellular resources or the replication and transcription mechanisms of the baculovirus. This interference could disrupt the efficacy and purity of the desired protein expression by the baculovirus. Obtaining rhabdovirus-free Sf cell lines is essential to avoid potential safety hazards associated with the production of recombinant proteins via BEVS from Sf-rhabdovirus-contaminated Sf cells (Maghodia et al., 2016). Therefore, detecting the presence of this virus in our newly

established cell line is crucial. In line with the research conducted by Ma in 2014 (Ma et al., 2014), primers were developed specifically to target the L protein-encoding gene of Sf-rhabdovirus (Supplementary Table S2). We employed a total of three sets of specific primers for PCR amplification. When the Mono1/2 primer was used, no observable amplification products were detected in either IOZCAS-Sf-1 of IPLB-Sf9 cells (Supplementary Fig. S1). With the Mono3/4 primer, no amplification product was observed in IPLB-Sf9 cells, but a band of approximately 730 bp was detected in IOZCAS-Sf-1 cells, although subsequent sequencing confirmed that this band did not correspond to the expected amplification product (Supplementary Fig. S1). Similarly, no amplification product was detected in IPLB-Sf9 cells with the Mono5/6 primer, but a band of 826 bp with the expected size was observed in IOZCAS-Sf-1 cells (Supplementary Fig. S1), which upon sequencing verification, did not correspond to a specific amplification product. Despite the absence of rhabdovirus-positive samples, our findings support the absence of rhabdovirus in both IOZCAS-Sf-1 and IPLB-Sf9 cells.

5. Conclusions

The establishment of the IOZCAS-Sf-1 cell line derived from *S. frugiperda* ovaries provides a valuable tool for advancing research on the biological aspects of *S. frugiperda* and developing effective pest management strategies. The unique characteristics of this cell line, such as slower growth but increased resistance to shear forces, make it suitable for large-scale cultivation and production. Its susceptibility to both AcMNPV and SfMNPV further enhances its potential use in toxicology studies, virus infection research, and anti-insect gene screening. These attributes provide valuable technical support and a solid scientific foundation for the management and control of *S. frugiperda*.

Data availability

All of the data generated during the current study are included in the manuscript or supplementary information. The *COX1* gene partial sequences obtained from IOZCAS-Sf-1, IPLB-Sf9, and hemocytes of *S. frugiperda* from the Yunnan population in China, were submitted to NCBI (GenBank accession numbers: PP727358, PP727359, PP727360) and ScienceDB (Accession link: https://doi.org/10.57760/sciencedb.15654).

Ethics statement

This study was conducted in accordance with the ethical principles of research and relevant guidelines and regulations. All of the data were collected and used for research purposes only.

Author contributions

Yan Tong: data curation, formal analysis, methodology, software, validation, visualization, writing-original draft. Wenyi Jin: formal analysis, investigation, software, visualization. Xuan Li: investigation, resources. Lin Guo: formal analysis. Gang Luo: investigation, resources. Qian Meng: data curation and software. Jihong Zhang: reviewing and editing. Qilian Qin: conceptualization, funding acquisition, project administration, supervision, reviewing and editing. Huan Zhang: conceptualization, data curation, funding acquisition, methodology, project administration, supervision, validation, writing-original draft, reviewing and editing.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2024.10.002.

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