Effect of Temperature Oscillation on Insect Cell Growth and Baculovirus Replication

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Received 29 December 1997/Accepted 1 April 1998

Temperature oscillation can enhance cell viability of sf9 insect cells and baculovirus production of occlusion bodies (OB) and extracellular virus (ECV) compared with constant temperature in stationary culture and suspension culture. The optimal oscillation range was 24 to 28°C. At this temperature oscillation, the viability of uninfected and infected sf9 cells can be maintained much longer than at 28°C. Although the rate of virus infection was a little low at 24 to 28°C, the final cell infectivity was similar to that at a constant temperature of 28°C. The production of OB was increased from 13.4 to 17.4/cell in stationary culture and from 13.9/cell to 18.1/cell in suspension culture. The titer of ECV was increased from 87 to 114 PFU/cell in stationary culture and from 79 to 114 PFU/cell in suspension culture.

MATERIALS AND METHODS

Cell line and virus stock. The sf9 insect cell line (Spodoptera frugiperda) was provided by the College of Life Sciences, Peking University. Cells were grown in TC-100 medium (Gibco) supplemented with NaHCO3 (0.5 g/liter)–10% (vol/vol) provided by the College of Life Sciences, Peking University. Cells were grown in

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RESULTS

Effect of temperature oscillation on uninfected insect cell growth. Figure 1 shows the growth of uninfected insect cells in stationary cultures with different temperature oscillations. The 28°C culture, the culture oscillating between 26 and 28°C, and the culture oscillating between 24 and 28°C had approximately the same maximal viable cell densities (18.4 × 10^6, 18.8 × 10^6, and 18.9 × 10^6 cells/ml, respectively). The maximal cell density was reached at the 4th day in the 28°C culture but was not reached until the 5th day in the culture oscillating between 26 and 28°C and the 6th day in the culture oscillating between 24 and 28°C. The cell growth phase in the culture oscillating between 24 and 28°C was about 2 days longer than that in the culture at a constant 28°C. The maximal cell densities of cultures oscillating between 22 and 28°C and those oscillating...
between 20 and 28°C were not as high as that of the 28°C culture (16.4 \times 10^5 and 14.3 \times 10^5 cells/ml versus 18.4 \times 10^5 cells/ml), whereas the viable cell density did not reduce at the 7th day in these two cultures. The effect of temperature oscillation on insect cell growth in suspension cultures is shown in Fig. 2. The culture with oscillation between 24 and 28°C gave the highest viable cell density (25.4 \times 10^5 cells/ml) among all the cultures, and its cell growth phase was 2 days longer than that at 28°C. These results indicated that temperature oscillation can prolong the cell growth phase. Oscillation between 24 and 28°C was optimal for promoting high cell viability without decreasing the maximal cell density.

Effect of temperature oscillation on infected insect cell growth. The growth of infected insect cells in stationary cultures at different temperature oscillations is shown in Fig. 3. The viability of infected cells declined quickly at 28°C and slightly more slowly with oscillation between 26 and 28°C at 1 day postinfection, whereas the viability of other cultures did not change much in the first 3 days postinfection. The culture with oscillation between 24 and 28°C at 3 days postinfection. This suggested that cultures with temperature oscillation can maintain high levels of cell viability, even postinfection. Results with suspension cultures were similar to those with stationary cultures (Fig. 4).

Temperature oscillation was beneficial for maintaining infected cell viability. When the lower limit of temperature oscillation was below 24°C, the viable cell density could be maintained for 3 days.

Effect of temperature oscillation on cell viability and virus infectivity. To investigate the effect of temperature oscillation on virus infection, cell viability and virus infectivity were examined in the 28°C culture, the culture with oscillation between 24 and 28°C, and the culture with oscillation between 20 and 28°C. Figures 5 and 6 show the cell viabilities and virus infectivities in stationary cultures and suspension cultures, respectively. There was much similarity between the stationary culture and the suspension culture, except the infection rate in the suspension culture was a little higher than that in the stationary culture. This can be explained by the fact that the movement of released virus particles from infected to noninfected cells in suspension culture is easier than that in stationary cultures (5). When the rate of virus infection was high, the cell viability dropped rapidly at 28°C. Compared with the culture at 28°C, the culture with oscillation between 24 and 28°C had a higher cell viability and a slightly lower rate of virus infection. In the culture with oscillation between 20 and 28°C, virus infectivity remained below 80% and cell viability was
nearly 40% at the 4th day postinfection. More time was probably needed to attain a higher infectivity in this oscillation culture.

Effect of temperature oscillation on virus production. Table 1 indicates the virus productions in both stationary and suspension cultures under different temperature conditions. Among all experiment conditions, the oscillation between 22 and 28°C was optimal for virus production in terms of the titer of ECV and the number of OB. The virus production in the culture with oscillation between 24 and 28°C was significantly higher than that of the 28°C culture by the t test for independent samples (Table 1). The titer of ECV was increased 18.4% on a volumetric basis and 31.0% on a cellular basis in the stationary culture. These increases were 23.0 and 44.3%, respectively, in the suspension culture. The number of OB was increased 16.7% on a volumetric basis and 30.0% on a cellular basis in the stationary culture, and these increases were 10.7 and 30.2%, respectively, in the suspension culture. The culture with oscillation between 26 and 28°C also produced more ECV and OB on a cellular basis than the culture at 28°C did. Compared with the 28°C culture, the virus production of the culture with oscillation between 22 and 28°C was no less on a cellular basis and less on a volumetric basis. The culture with oscillation between 20 and 28°C culture produced less virus than the 28°C culture at the 4th day postinfection. Since the virus infectivity was below 80% at that time, the virus production in the former culture might be increased if its time were prolonged.

**DISCUSSION**

Our results show that temperature oscillation can prolong the cell growth phase of uninfected and infected sf9 cells in stationary and suspension cultures. The optimal oscillation for promoting a long cell growth phase without decreasing the maximal cell density was between 24 and 28°C. At temperatures below 22°C, cells grew too slowly (8) and did not reach a cell density as high as that reached in the 28°C culture. An additional reason for the long phase of cell viability postinfection was the low infection rate due to the low temperature (8). As quick infection at high temperatures leads to quick lysis, implementing a suitable temperature oscillation was able to increase baculovirus production. There are two possible explanations for this: either infected insect cells survive longer at temperatures oscillating from low to high so that they can produce more virus or the slow infection that results from temperature oscillation might leave some viable cells to divide, causing secondary infection (6, 7) and thus increasing virus production.

**ACKNOWLEDGMENT**

We thank D. E. Lynn (USDA, ARS, Beltsville, Md.) for critical review of the manuscript and for helpful advice.

**REFERENCES**