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In Vitro Effect of Insect Hemolymph on the Production of Polyhedra of a Nuclear Polyhedrosis Virus¹

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Although the silkworm cell line, SES-BoMo-15A (INOUE and MITSUHASHI, 1988), can be subcultured in a low-cost Mitsuhashi and Maramorosch (MM) medium containing 3% fetal bovine serum (FBS) (MM·3%FBS; MITSUHASHI and MARAMOROSCH, 1964), these cells produce only a negligible number of polyhedra following the infection with *Bombyx mori* nuclear polyhedrosis virus (BmNPV) (WATANABE et al., 1988). When the cells in MM·3%FBS medium are transferred into MGM-443 medium (MITSUHASHI, 1984) containing 3% or 10% FBS, the number of cells producing polyhedra increases significantly, whereas the production of polyhedra increases little in cells trans-

ferred into MM medium containing 10% FBS (INOUE et al., 1988; WATANABE et al., 1988). These results suggest that the MM medium has inadequate nutrition for efficient virus multiplication and/or polyhedra formation in SES-BoMo-15A cells infected with BmNPV. In the present study, we report that the silkworm hemolymph promotes markedly the production of polyhedra in BoMo-15A cells cultured in MM·3%FBS medium.

The hemolymph of 6-day-old 5th instar larvae reared on mulberry leaves were collected in an ice-cold test tube and then heated at 60°C for 1 hr in a water bath. The hemolymph was centrifuged at 3,500 rpm for 20 min and the supernatant was stored at –20°C. The thawed hemolymph was added at appropriate amounts to the MM medium containing 3% FBS or no FBS, and the final medium was passed through a 450 nm pore size filter.

The cells cultured in the MM·3%FBS medium divided equally into 3 tubes, and centrifuged at 1,500 rpm for 2 min. The pelleted cells were resuspended each in 4 ml of fresh medium containing, 1) MM·3%FBS, 2) MM·3%FBS·10% hemolymph or 3) MM·10% hemolymph, at a cell density of about 2×10^5 cells/ml, and cultured at 25°C in a 50 ml plastic culture flask (Falcon Co.). On day 2 after cell seeding, the cultures were inoculated with 100 μ l of BmNPV inoculum with a titer of 5×10^6

Table 1. Effect of hemolymph on the formation of polyhedra

Media	Test No.	No. cells observed	No. cells with polyhedra	Percentage (%)
MM·3%FBS	I	1701	0	0.0
	II	1670	0	0.0
MM·3%FBS·10%HE	I	1647	379	23.0
	II	1633	461	28.2
MM·10%HE	I	1590	397	25.0
	II	1681	555	33.0

Cells were observed on day 4 (I) or 5 (II) post BmNPV inoculation.

HE means hemolymph.

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Fig. 1. Polyhedra formation of BmNPV in cells maintained in MM·3%FBS medium containing silkworm hemolymph. a: Absence of polyhedra in cells in the medium without hemolymph. b: Polyhedra formed in cells in the medium with 10% hemolymph. c: Virus rods occluded in polyhedra. Bars show 10 μ m (a, b) and 1 μ m (c).

Table 2. Effect of hemolymph concentration on the formation of BmNPV polyhedra

Hemolymph concentration (%)	No. cells observed	No. cells with polyhedra	Percentage (%)
10	1870	592	31.7
5	1861	320	17.2
3	1954	189	9.7
1	1971	69	3.5
0	1790	0	0.0

Cells were observed on day 6 post BmNPV inoculation.

plaque forming units/ml. The BmNPV inoculum was a 50 times dilution of a supernatant obtained by centrifuging the hemolymph of diseased larvae at 3,500 rpm for 20 min at 4°C. The cells were observed for the presence of polyhedra at the 4th, 5th or 6th days post BmNPV inoculation with an inverted microscope.

The addition of hemolymph to the MM·3%FBS medium accelerated the formation of BmNPV polyhedra (Table 1, Fig. 1a, b). Electron microscopic observation showed the presence of virus rods in the polyhedra (Fig. 1c). The same effect of hemolymph for polyhedra production was observed for cells in the MM medium containing no FBS. In order to determine the effect of hemo-

lymph concentration on polyhedra formation, cells in MM·3%FBS medium were divided into 5 flasks and to each flask was added fresh MM media containing hemolymph at 10%, 5%, 3%, 1% and 0%, respectively. The flasks were cultured for 2 days at 25°C and then inoculated with BmNPV. The formation of polyhedra was observed on day 6 post virus inoculation. As shown in Table 2, the enhancing effect of hemolymph on the formation of polyhedra depended on the hemolymph concentration. This finding suggests the existence of enhancing factor(s) in the hemolymph.

GARDINER and STOCKDALE (1975) had substituted fetal calf serum and tryptose broth in place of *B. mori* hemolymph in order to develop

TC-7 and TC-10 media, in which the cells of *Spodoptera frugiperda* replicated and could be infected with SfNPV. We have shown that the silkworm hemolymph enhances and promotes the formation and development of NPV polyhedra in the cell line of *B. mori*. There is a need for a culture medium suitable for the propagation of insect viruses and the silkworm hemolymph can be used as a supplement to increase the propagation of NPV polyhedra in cell culture.

We are attempting to identify the factor(s) in the silkworm hemolymph that enhances the production of virus polyhedra and to determine whether this hemolymph will also enhance the production of other baculoviruses in other cell lines.

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Effect of Dung Conditioning by *Onthophagus lenzii* HAROLD on Oviposition of *Liatongus phanaeoides* WESTWOOD (Coleoptera: Scarabaeidae)¹

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The difference in resource utilization patterns among species with similar life modes can play an important role in coexistence of species (e.g. PRICE, 1984). However, this difference may influence reproduction of species.

The two dung beetle species, *Onthophagus lenzii* HAROLD and *Liatongus phanaeoides* WESTWOOD, have similar oviposition modes and periods (YASUDA, 1986). Adults of *L. phanaeoides* utilize old dung pats as well as fresh ones for their food and oviposition sites. Old pats often have already been utilized by many coprophagous beetle species, particularly, *O. lenzii* (YASUDA, 1987). I tested whether *L. pha-*

naeoides laid more eggs in fresh pats or in pats conditioned by *O. lenzii*.

MATERIALS AND METHODS

Dung conditioning was categorized as follows: (1) Fresh pat; cow dung was collected from a farmhouse within 4 hours after deposition and was artificially made into a dung pat (1 kg), (2) 3-day-old pat; a fresh pat in an experimental container (Fig. 1) was set in a study field for 3 days, (3) *O. lenzii*-conditioned pat; after the fresh pat in each

Fig. 1. Experimental container. a: 1 kg dung pat, b1: plastic basket, b2: plastic basket including soil, c: plastic ring.

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