数品種の家蚕胚組織から新たに樹立された細胞株の特徴

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Characteristics of cell lines established from embryonic tissues of several races of the silkworm, *Bombyx mori*, cultured *in vitro*

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Embryonic tissues from the Japanese, the Chinese, the European and the tropical and mutant strains of the silkworm, *Bombyx mori*, were cultured *in vitro*. Migration and proliferation of the cells in primary culture varied with the silkworm strains. The characteristics of two newly established cell lines designated as SES-BoMo-C129 and SES-BoMo-J125 were examined. The former consisted of five different kinds of cells, but the latter consisted of a few kinds of cells. The population doubling times ranges from 3.0 to 3.5 days. Two cell lines consisted of cells with 91-110 chromosomes mainly. Optimum conditions of culture for both cell lines were observed at 28°C and cell growth was optimum in the culture medium containing 10% fetal bovine serum.

In general, the silkworm, *B. mori*, has been geographically classified into the Japanese strain, the Chinese strain, the European strain, the tropical strain, and so on. These strains were characterized by the cocoon shape, cocoon color, egg color, voltinism and moltinism. The isozyme pattern of amylase, phosphatase and esterase of the strains was examined by Matsumura (1951) and Yoshitake (1968).

Successful culture of silkworm cells was first achieved by Trager (1935). At present, several cell lines of the silkworm have been established, ie. the cell line established by Grace (1967), S.P.C.Bm 36 (Quiot, 1982), Bm-N (Volkman and Goldsmith, 1982), SES-BoMo-15A (Inoue and Mitsuhashi, 1984) and SES-BmN-e 21 & SES-BmN-1 30 (Ninaki, 1987).

In the present study, the phases of cell migration from embryonic tissues of 15 geographical silkworm strains in the primary culture were examined and the characteristics of two cell lines newly established were studied.

Materials and Methods

Silkworm strains: The silkworm strains used here were as follows: three Japanese strains "J125", "J137" and "J145", five Chinese strains "C25", "C108", "C129", "C140" and "C146", one European strain "E18", one Tropical strain "Cambodge", five mutant strains "*U*", "*Ia*", "*rb*", "*pnd*" and "*pe, re*" (Glossary of Sericultural Science, 1979). The eggs, 20 hours after oviposition, were treated with hydrochloride acid (specific gravity 1.075) for 5 minutes at 46°C in order to avoid the diapause and incubated at 25°C. The embryos at the 21 th development stage (embryonic reversal stage, Takami and Kitazawa, 1960) were prepared for cell culture.

Cell culture: Eggs were surface-sterilized by submersion in 70% ethyl alcohol for 5 minutes, washed in sterile distilled water twice and trans-

ferred into Carlson's fluid (Carlson, 1946). The embryos were dissected into several parts and they were placed in a Falcon type 50 ml culture flask containing MGM 448 culture medium with 10% fetal bovine serum (FBS) (Mitsuhashi, 1984). The culture flasks were maintained at 25°C and half of the medium was renewed once a week during the primary cultre.

Karyology: Karyo types of cells which continuously proliferated were examined by the procedure of Earley (Earley 1975), using a pretreatment of 0.50 μg colchicine/ml. The cells were smeared on a glass slide and stained with Giemsa. Chromosomes were counted in fifty cells.

Growth curve of cells: After the subculture, cells that continuously proliferated were cultured at 15°C, 18°C, 25°C, 28°C, 33°C and 36°C, respectively. Also, cells were cultured in the MGM 448 media with a FBS concentration of 0%, 2%, 5%, 10% and 20%, respectively. The number of cells in each flask was counted by using a hemocytocounting chamber (Bürker-Türk) every day.

Results and Discussion

Cell migration and proliferation in the primary culture: Cell migration from the embryo tissues was observed within 24 hours after the initiation of the culture. However, the migration phase of the cells varied with the silkworm strains. As shown in Table 1, cell migration was very active in the first group. The epithelial-like cells migrated actively for about one month and the fibroblast-like cells formed networks quickly. In the second group, cell migration was not active during the first month, but became gradually active in the next month. The cells migrating from "Cambodge", "E18" and mutant "*Ia*" died in a comparatively short period of time and the cells migrating from the strains of "C25", "C108", "C140", "C146", "J145", "*pnd*" and "*U*" proliferated for a limited period of time and then died 4 months after the onset of the cultures. The cells migrating from the Japanese strain "J125" and the Chinese strain "C129" proliferated actively from 5 months after the culture, and these cells could be subcultured. The cells were exfoliated from flasks by pipetting. The cells were gradually detached from the base of the flasks and floated in the medium. These cells proliferated actively and were subcultured with a 1:2 split ratio. At about 170 days for "J125", and at about 150 days for "C129" after the onset of the culture, the number of subcultures reached 70 (May, 1987) and the cell lines which proliferated continuously were designated as SES-BoMo-J125 and SES-BoMo-C129, respectively. SES-BoMo-J125 was composed of cells with a diameter of 20.0±30.3 μm (Fig. 1), whereas SES-BoMo-C129 consisted of five kinds of cells, ie. the type I (23.10±1.0 μm in diameter, 64.9% of the total cells), type II (16.8 ±0.7 μm in diameter, 7.4% of the total cells), type III (32.4±1.7 μm in diameter, 5.8% of the total cells), elongated cells (20.2% of the total cells) and aggregated cells (1.6% of the total cells) (Fig. 2).

Table 1. Chracteristics of cell migration in early primary culture

Cell migration	strains
Active	C25, C108, C125, C129, C146, Ia, U, pnd, pe re, rb
Inactive	J125, J137, J145, E18, Cambodge

C: Chinese strain J: Japanese strain
E: European strain

The geographical strains of the silkworm, *Bombyx mori* were classified by the analysis of the amylase of the digestive juice, the phosphatase of hemolymph and the esterase of the epidermis, and so on. The cell migration from embryonic tissues of 15 geographical strains in

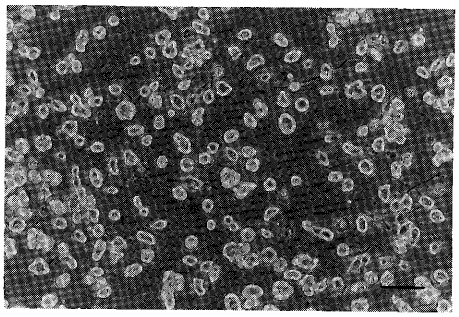

Fig. 1. Cells of SES-BoMo-J125. The bar represents 100 μm.

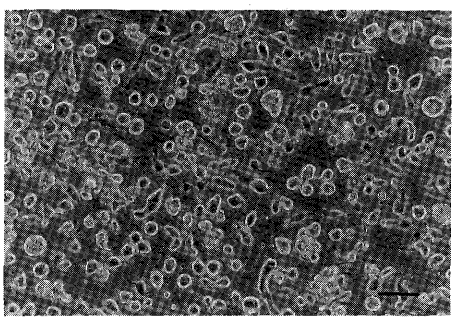

Fig. 2. Cells of SES-BoMo-C129. The bar represents 100 μm.

the embryonic reversal stage is currently being examined, leading to a classification into an active group and inactive group of cells. The Japanese strains showed an active type cell of migration from the explants in the early period of the primary culture. Thereafter cell division became inactive, while the Chinese strains showed an inactive type of cell migration in the early period of the primary culture.

Karyotype: After 70 subcultures of the SES-BoMo-J125 and SES-BoMo-C129 cell lines, the chromosome number of the cells was examined. As shown in Fig. 3, the chromosome number of the SES-BoMo-J125 line ranged from 31 to 230 whereas that of the SES-BoMo-C129 line ranged from 31 to 310. The mean chromosome number was in the range of 91–110 for both cell lines. The SES-BoMo-C129 and SES-BoMo-J125 cell lines showed polyploidy, suggesting the presence of chromosome number as that of the *Bombyx mori* cell line of Grace (1967) and that of lepidopteran cells containing microchromosomes with diffuse centromeres (Ennis and Sohi, 1976).

Cell growth at various temperatures and concentrations of FBS: The growth curve of the SES-BoMo-J125 cell line showed some tendency of cell proliferation at temperature ranging for 18°C to 36°C (Fig. 4), whereas the cells of the SES-BoMo-C129 cell line proliferated with the increase of the temperature (Fig. 5). Both cell lines showed optimum conditions at 28°C. The population doubling times were 3.0 days for the SES-BoMo-J125 cell line and 3.5 days for the SES-BoMo-C129 cell line, respectively. However the proliferation of the cell lines did not in-

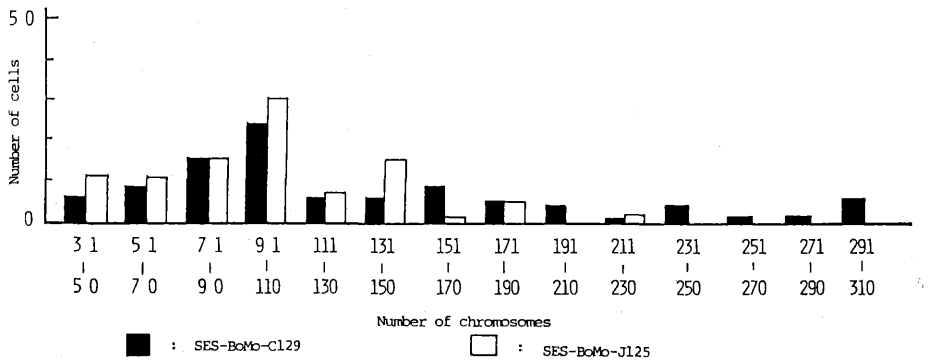

Fig. 3. Cells with a different number of chromosomes in the cell lines.

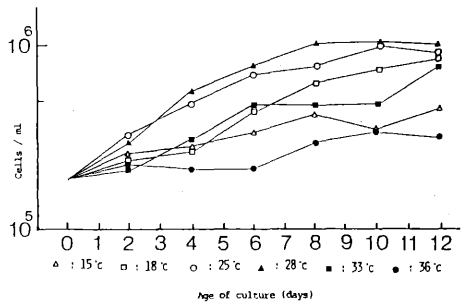

Fig. 4. Effect of temperature on cell proliferation. (SES-BoMo-J125)

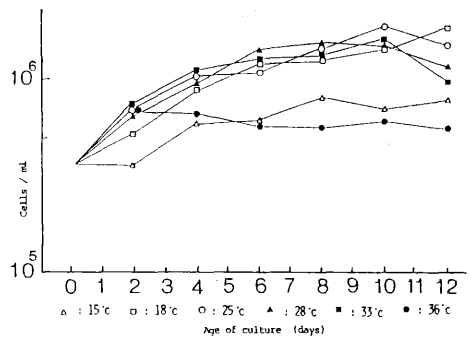

Fig. 5. Effect of temperature on cell proliferation. (SES-BoMo-C129)

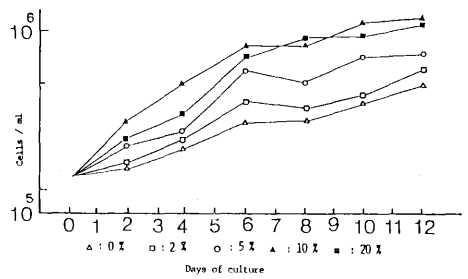

Fig. 6. Effect of FBS concentration in the culture medium on cell proliferation. (SES-BoMo-J125)

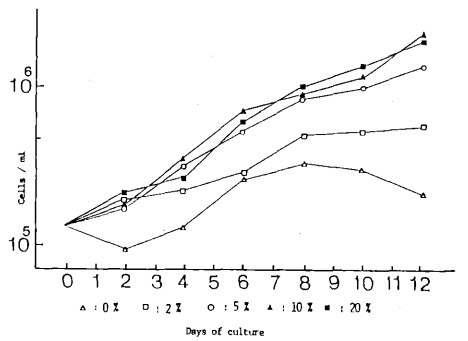

Fig. 7. Effect of FBS concentration in the culture medium on cell proliferation. (SES-BoMo-C129)

crease at 15°C and 36°C. The SES-BoMo-J125 and the SES-BoMo-C129 cell lines exhibited similar growth rates in the media containing 5% FBS, 10% FBS and 20% FBS, while optimum cell growth was observed at 10% FBS (Fig. 6, 7). In both cell lines, the cells were able to grow continuously at the concentration of 2% FBS. When FBS was omitted from the medium, the cells grew for ten days, and subsequently no further multiplication occurred.

Fetal bovine serum is most often used at concentrations of 5% to 20% in the insect cell culture medium. The optimum concentration of FBS in the medium for cell growth varied with the cell lines. The cells from *A. eucalypti* and *B. mori* multiplied most rapidly in the medium with 5% FBS and 20% FBS, whereas 30% FBS caused a reduction in cell growth (Sohi and Smith, 1970). The current two cell lines (SES-BoMo-C129 and SES-BoMo-J125) multiplied actively in the medium with 10% FBS and the cells grew continuously in the culture medium with 2% FBS or 20% FBS.

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今西重雄・大槻良樹：数品種の家蚕胚組織から新たに樹立された細胞株の特徴

日本種，中国種，欧州種，熱帯種及び突然変異種の家蚕の胚組織を *in vitro* で培養し，品種間差異を検討した。初代培養期間中，胚組織から細胞遊離及びそれらの遊離細胞の増殖は日本種及び中国種で異なり，培養の前期または後期に活発に遊離，増殖するタイプに区別できた。新たに樹立した細胞株は SES-BoMo-C129 及び SES-BoMo-J125 と名づけた。前者は5種類の細胞型から構成され後者は2～3種類の細胞型から構成されていた。両細胞株の細胞倍加日数は3.0～3.5日間であり，染色体数は細胞株ごとに異なっていたが，主に91～110本の範囲にあった。至適細胞増殖条件は両細胞株とも培養温度は 28°C，培地中の血清濃度は 10% であることが分った。