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Novel Culture Techniques to Establish New Insect Cell Lines

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About 500 insect cell lines have been established over the 35 years. The cell lines of *Bombyx mori* (Bm), *Spodoptera frugiperda* and *Trichoplusia ni* are used as a tool of baculovirus gene expression system. And cell lines are very significant materials in molecular biology, too. However, culture techniques are not adequately established for most insect species and their tissues. In this study, we developed two types of novel culture medium and also cell matrix. The cells that migrated from the tissues of several insect species increased easily by nobel medium of MX medium and SX medium. MX is better for growth of the cells derived from *Lepidoptera, Coleoptera, Hemiptera.* SX is better for the growth of *Diptera*'s cells. Notably MX30 medium containing 30% of fetal bovine serum (FBS) accelerated a migration and multiplication of the cells, and shortened the primary culture term. Now we are continuing the primary culture of *Plautia stali* cells derived from embryo, of *Anomala cuprea* cells from fatty body, of *Agrius convolvuli* cells from fatty body, of *Bombyx mandarina* cells from fatty body and of *Bombyx mori* cells from ovary and testis. *Culicoides oxystoma* cells from embryo are now growing in SX30 medium. Water soluble chitin, N-trimethylchitosan and Sulfonated that modified chitins extracted from pupa's skins of *Bombyx mori*, were good materials as extra cellular matrix. Especially, water soluble chitin promoted the cell migration in an extreme low density of 0.001% W/V. These chitosans seemed to have a role to act as an adhesive between cell and culture bed. But chitosans have tissue specificity, i.e. fat body tissue does not have a strong adhesive reaction with chitosans. A novel insect primary culture method by using developed medium and extra cellular matrix provided stimulatory factors to aid growth of the primary culture cells. By this technique, we established a nobel *Bombyx mori* cell line. This cell line can be cultured with the medium of an extreme low density of FBS and shows high susceptibility to BmNPV. We can recommend an ideal tool of BmNPV baculovirus gene expression vector system.