

## Development of Insect Cell Lines and Their Utilization

Shigeo Imanishi

*Insect Biotechnology and Sericology Department, NIAS, Tsukuba, Ibaraki 305-8634 Japan*

While over 500 insect cell lines have been established over the past 35 years, most were developed using generalized tissues (such as embryos, or ovaries). Cell lines are very significant materials in molecular biology and pathology. However, cultural technique is not adequately established for all kind of insect species and their tissues. And it takes over one year till establishment as a cultured cell line. Here I developed two types of novel culture medium and unique culture method of cell extracellular matrix. New mediums, such as MX and SX, can easily increase cells that migrated from several kinds of insect species and of tissues. MX medium is mainly better for Lepidoptera, Coleoptera, Hemiptera and their tissues. SX medium is better for the growth of Diptera's cells. Especially, MX30, including 30% volume of FBS in the MX medium, could accelerate a migration and multiplication of the cells from testis tissues of *Bombyx mori*, and could shorten the primary culture term in several months when used by a small scale culture bed. Now we are continuing the subculture 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 fatty body, ovary, embryo, hemocytes and testis. *Culicoides oxystoma* cells from embryo are now growing in SX30 medium. Water soluble chitin that modified chitins extracted from pupa's skins of *Bombyx mori*, were good materials as extra cellular matrix. Especially, water soluble chitin promoted cell migration from gonad tissues, in a low density of 0.001%-1% W/V. Chitin seemed to have a role to act as an adhesive between cell and culture bed. But chitin had tissue specificity, i.e. fatty body tissue did not have a strong adhesive reaction. A novel insect primary culture method by using newly developed medium and extra cellular matrix provides stimulatory factors to aid growth of the primary culture cells.

The established cell lines are making wonderful researches. One cell line is being used as a large scale culture *in vitro*. The other cell lines are confirmed that *Bombyx* cell line can keep Wolbachia, some *Bombyx* cell lines maintain a certain kind of plant disease viruses, and one cell line reacts typically to insect hormone.

We are researching together by using experimental tools of the cultured cells.