Cultivation of Intracellular Yeast-like Organisms in the Smaller Brown Planthopper, *Laodelphax striatellus* FALLÉN (Hemiptera, Delphacidae)[1]

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The presence of yeast-like organisms (YLO) in the cytoplasm of mycetocytes of planthoppers has been known (BUCHNER, 1953). In the smaller brown planthopper, *Laodelphax striatellus*, such mycetocytes were found in fat bodies situated in the abdomen. The YLO have been thought to be symbiotic in nature, although their role has not been clarified. It has been generally recognized that in the body of adult females, these organisms are transferred from fat bodies to ovaries, and penetrate into the egg as a symbiote ball via the ovarial pedicel. In this manner they are transferred from generation to generation through ovaries.

In order to obtain growing YLO *in vitro*, attempts were made to cultivate those taken from the egg of *L. striatellus*. The wild type of *L. striatellus* newly collected from fields as well as a red eye mutant which was isolated in this laboratory were used. Eggs of the same stage were obtained by MITSUHASHI's method (MITSUHASHI, 1970). The eggs were first surface sterilized by submersion in 70% ethyl alcohol for 1 minute, and then washed in sterilized distilled water. The eggs were transferred into the culture medium, placed in the culture vessel, and the chorions were removed with the aid of fine needles under a binocular dissecting microscope. The embryo and yolk were then dispersed in the medium. This resulted in even dispersion of YLO on the bottom of the culture vessel. The vessel was the same as that used for leafhopper tissue culture MITSUHASHI and MARAMOROSCH, 1964); the culture medium was MGM-401, which was also the medium formulated for leafhopper tissue culture (MITSUHASHI, 1975). The culture was maintained at 25°C. When growth of YLO was obtained, they were transferred into square bottles (65 mm × 40 mm × 25 mm), or onto an agar plate containing MGM-401.

Some YLO were in the stage of budding when taken out of eggs. In the culture, YLO multiplied by budding in some cases (Fig. 1a), but in most instances they grew by means of extending hyphae (Fig. 1b). Occasionally both types of growth occurred simultaneously (Fig. 1c). The hyphae developed further and formed mycelia. These

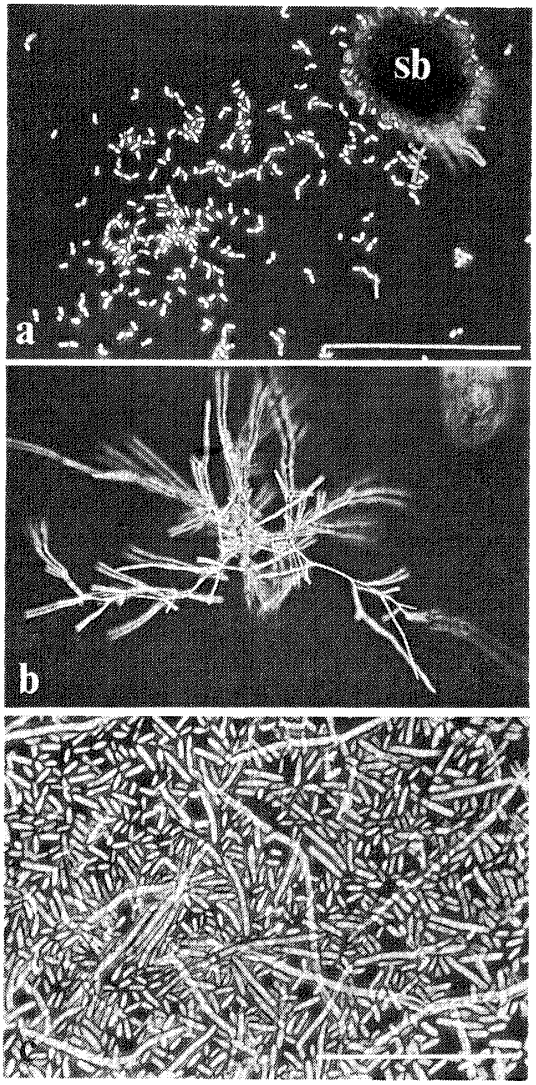

Fig. 1. a : Multiplication of YLO by budding. Two days after onset of cultivation. sb, symbiote ball. Line indicates 200 μm. b : Multiplication of YLO by extending hyphae. Two days after onset of cultivation. Magnification is the same as a. c : Multiplication of YLO by budding and extending hypae. Five days after onset of cultivation. Line indicates 100 μm.

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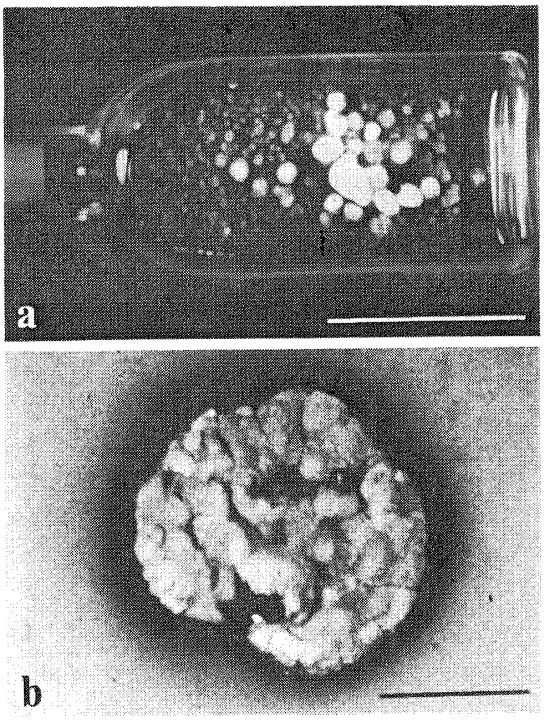

Fig. 2. a : Mycelia. Small mycelia are shown mostly in the medium, while large ones are seen floating on the surface of the medium. Line indicates 4.0 cm. b : A large colony formed on agar plate containing MGM-401 medium, showing darkening of the agar at the periphery of the colony. Line indicates 1.0 cm.

mycelia appeared grey in color when in the medium, while their appearance was a velvet-like white when found floating on the medium and in contact with air. These mycelia increased in number as cultivation progressed (Fig. 2a). When a mycelium was transferred onto an agar plate, it increased in size, and formed a dark pigment in agar which contacted with YLO (Fig. 2b). In old cultures on agar plates, the mycelium developed into a giant colony which exhibited a velvet-like white central area and marginal periphery that was dark and separated from the agar.

It is often very difficult to prove that the microorganisms grown in a culture actually originated from symbiotes of insects, rather than from extraneous contamination. In the present study the cultivated microorganisms originated definitely from intracelluar YLO of *L. striatellus*. This was demonstrated as follows : A symbiote ball which consisted of a mass of YLO was isolated from an egg of *L. striatellus*, and cultivated *in vitro*.

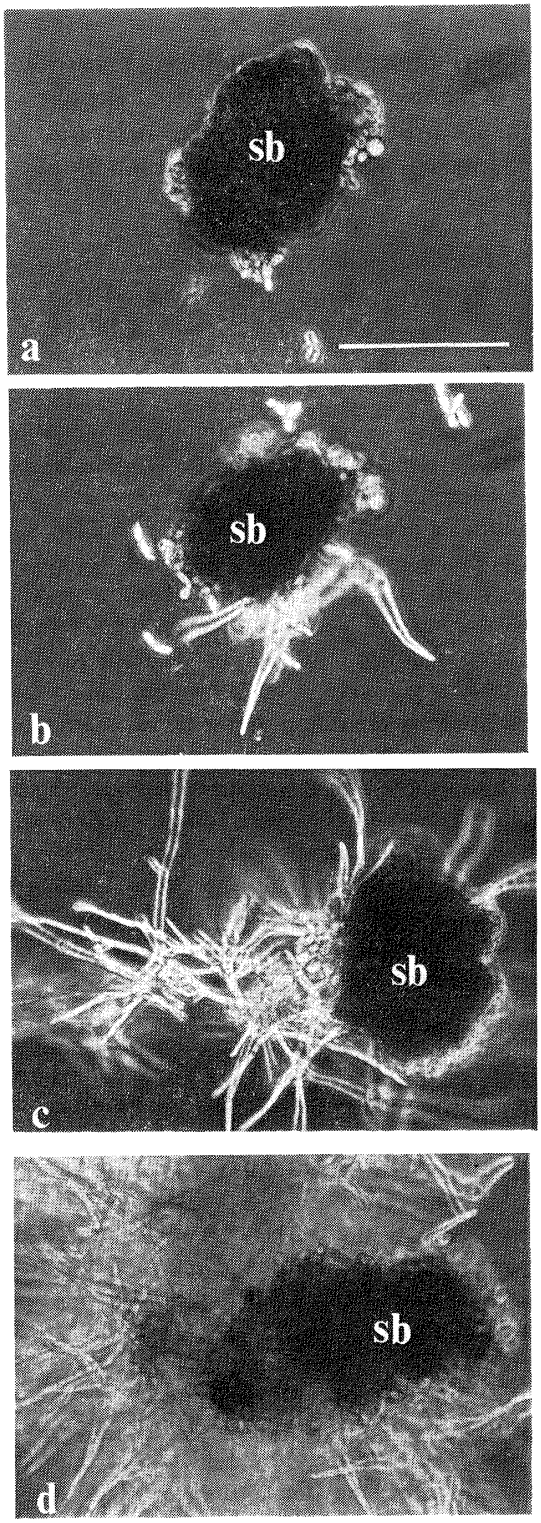

Fig. 3. Sequential photographs of the same symbiote ball in culture. a : Commencement of culture. b : One day after cultivation. c : Two days after cultivation. d : Three days after cultivation. sb, symbiote ball. Line indicates 100 μm.

Sequential photography gave evidence for the multiplication of YLO *in vitro*. Hyphae appeared from the symbiote ball one day after the onset of cultivation, and developed with advancing cultivation (Fig. 3).

The effects of some antibiotics and antiseptics on the growth of YLO *in vitro* were also examined. Bacterial antibiotics, such as kanamycin, streptomycin, sarcomycin, novobiocin, and penicillin showed practically no effects. Mycostatin, actidion, and blastocidin, which are fungal antibiotics inhibited the growth of YLO at concentrations of 10^{-3} to 10^{-2} mg per ml. Some antiseptics such as methyl-p-hydroxybenzoate, methyl-naphtoquinone, and merzonine, also inhibited the growth of YLO at concentrations of 10^{-3} to 10^{-2} mg per ml.

Variation and change in growth ability of YLO were found *in vitro*. The YLO from *L. striatellus* newly collected from fields grew well *in vitro*, while those from the red eye mutant, failed to grow under similar conditions, although the eggs of both strains contained similar amount of YLO. The YLO from wild type planthoppers, however, were unable to grow *in vitro* after the planthoppers were reared for some generations on rice seedlings in small glass tubes. This phenomenon requires further confirmation. Furthermore, variation in *in vitro* growth ability of YLO from planthoppers in different districts should also be investigated.

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