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MORPHOLOGY AND REPRODUCTION OF YEASTLIKE ENDOSYMBIOTES OF THE WHITEBACKED PLANTHOPPER, *SOGATELLA FURCIFERA* (HORVATH) (HOMOPTERA: DELPHACIDAE)[1]

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ABSTRACT

The dominantly occurring yeastlike endosymbiotes in the whitebacked planthopper, *Sogatella furcifera* (Horvath), were examined in hemolymph and fat tissue smears stained with crystal violet. The endosymbiotes, mostly mononucleated, were oval or elliptical cells (8.57 x 2.28 u) with two or three attenuated termini. A single *S. furcifera* female possessed more than a thousand yeastlike symbiotes which propagated by single or double budding.

Key words: *Sogatella furcifera*, whitebacked planthopper, yeastlike endosymbiotes.

INTRODUCTION

Symbiotic associations exist between insect hosts and microorganisms. The internal flora of microorganisms, called intracellular endosymbiotes, occur within certain tissues of insect bodies. They are important for the host's general body functions, such as nutrition or metabolism, and are usually transmitted to the next host generation trans-ovarially (Buchner, 1965).The intracelluar symbiotes of some homopterans have been well investigated. Aphids and leafhoppers carry symbiotes in specialized structures called mycetomes, while planthoppers harbor them in specialized cells or mycetocytes in fat bodies (Nasu, 1963, Noda, 1977).The endosymbiotes of the rice planthoppers, *Nilaparvata lugens* (Stal), *Laodelphax striatellus* Fallen, and *Sogatella furcifera* (Horvath) are yeastlike (Nasu, et al. 1981). The cellular morphology and the mode of reproduction of these endosymbiotes have not been described. We describe the morphological and reproductive features of yeastlike endosymbiotes of the whitebacked planthopper, *S. furcifera*, an important pest of rice in South and Southeast Asia (Suenaga, 1963).

MATERIALS AND METHODS

Two-day-old, brachypterous *S. furcifera* females were collected from a stock culture of the insect maintained on 40-45-d-old susceptible 'Taichung Native 1' rice plants. The individuals were lightly anesthetized with carbon-dioxide gas and then stored for 24 h in 5 ml of Hank's basic salt solution (0.14 g KCl, 6.5 g NaCl, 0.12 g CaCl, 0.2 g $NaHCO_3$, and 1 liter of distilled water) in vials. The abdomen of each insect was separated from the head and thorax and the abdominal contents

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squeezed out in a drop of the salt solution. The abdominal exoskeleton and viscera were discarded, leaving only the smears of hemolymph and fat bodies. A drop of 1% crystal violet stained the smear preparations. Using a clean glass slide, at 45° inclination, the preparation was immediately spread thinly and uniformly on the slide. Excess smear was spread on another side. The prepared slides were air-dried for 24 h. Excess stain was removed by immersing the slide in a beaker with distilled water. Again, the slides were airdried for 2 h. They were mounted permanently in Canada balsam and examined under oil immersion objective (x 1250) of a Leitz Dialux 22 research microscope. Mycetocytes were measured using calibrated built-in micrometer and endosymbiotes were photomicrographed. A total of 50 *S. furcifera* females was examined for the endosymbiote study.

RESULTS AND DISCUSSION

S. furcifera harbored intracellular symbiotes, comprising colonies of rod-shaped bacteria and yeastlike symbiotes. The dominantly occurring microorganisms were the yeastlike endosymbiotes (**Fig. 1**). They were obtained from smears of the hemoplymph and abdominal fat tissues. A female *S. furcifera* possessed more than 1000 yeastlike endosymbiotes. As the ovary also housed these mircoorganisms, their transovarial transmission to progenies was likely. Transovarial transmission of endosymbiotes in *N. lugens* and *L. striatellus* is known (Nasu, 1963, Noda, 1974, 1977).

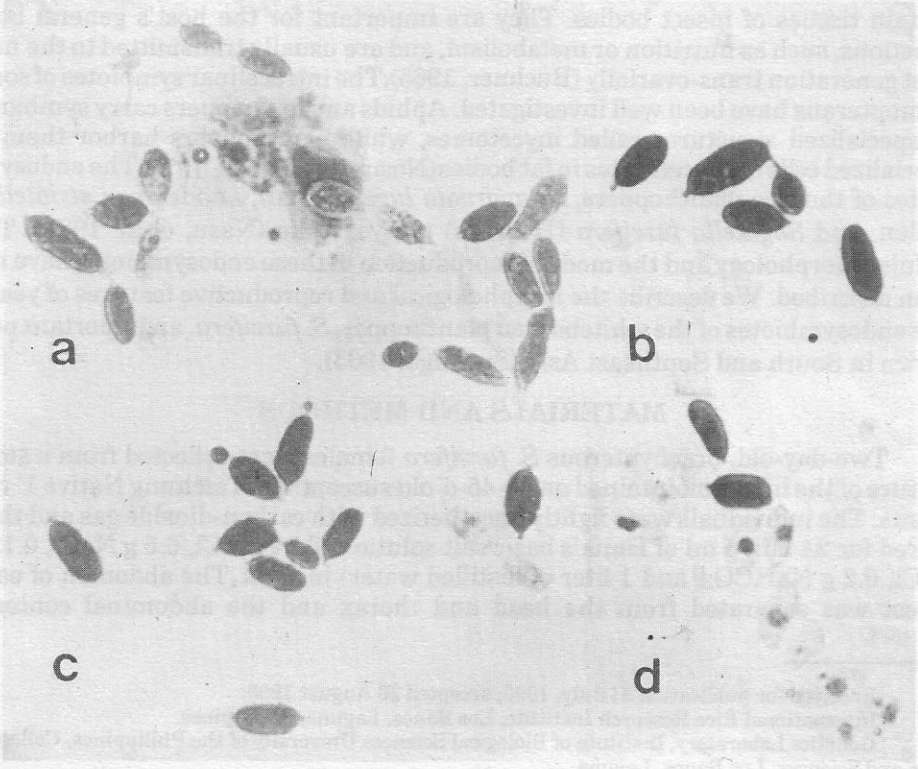

Fig. 1. Nonreproductive (a,c) and reproductive (b,d) forms of yeastlike endosymbiotes of the female *S. furcifera*, Magnification, 1000 x (oil immersion).

The yeastlike endosymbiotes had variable shapes; 68% were ovoid cells with one or both ends somewhat attenuated, while others were more or less elliptical with one to three minute termini. The termini were determined to be the possible sites for budding. Symbiote populations of *S. furcifera* comprised both reproductive (25%) and non-reproductive (75%) forms. The isolated non-reproductive forms averaged 8.57 by 2.28 u. The protoplasm was surrounded by two thin membranes, the outer being dense and hyaline. The cytoplasm had diffused metachromatic granules, a few small vacuoles (0.1 - 5.0 u diam) and one to two big vacuoles (1 u diam). A great majority of the cells were mononucleated; the nucleus was usually centrally located but in some cases it was adhered to the peripheral membranes. Some cells also contained two nuclei - one macro - and one micronuclei (**Fig. 1**.

The endosymbiotes multiplied through budding in two ways: 1.the single terminal budding (**Fig. 2a-j**), and 2. the double budding, comprising terminal plus somewhat lateral buddings (**Fig. 3a-d**). Single budding occurred more frequently (69%) than double budding (31%). The initial buds that were produced were circular (1.43 u diam) (**Fig. 2g**). Then, they became oval or elliptical (5.99 x 2 u), with pointed end toward the mother cell and attached by a thin, long neck (**Fig. 2h-j**). The buds persisted until fully grown. Usually, the bud detached from the mother cell when they reached an average body dimension of 6 by 2 u. Under normal conditions, about 25% of the yeastlike endosymbiotes in each *S. furcifera* female observed were going through the budding process. Kusumi et al. (1979) isolated two yeastlike symbiotes from the mycetocytes of eggs and fat bodies of *N. lugens* and *L. striatellus*, but we identified both rod-shaped bacteria and yeastlike endomsymbiotes in the smears of the hemolymph and fatty tissue of *S. furcifera* abdomen.

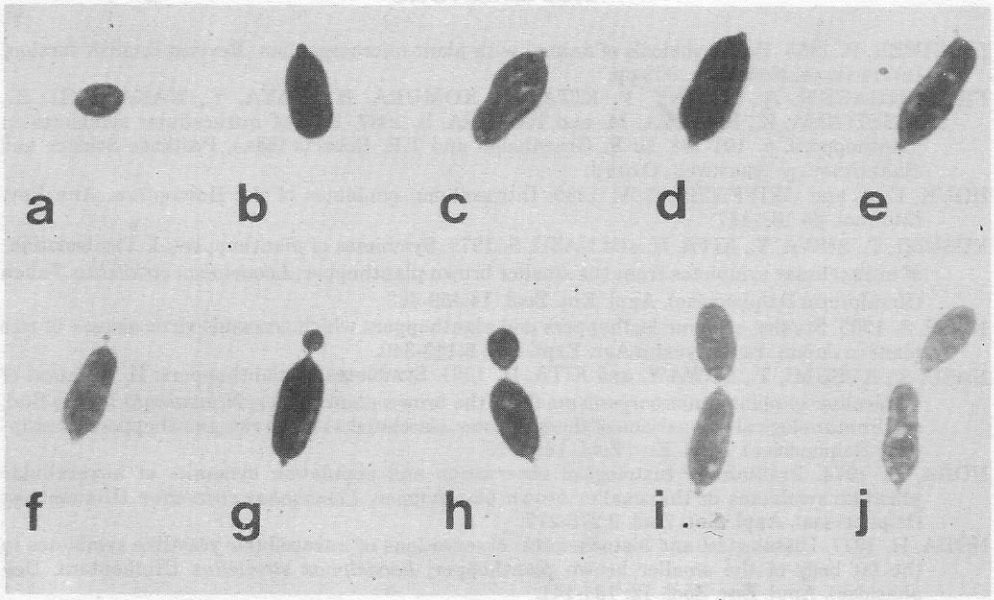

Fig. 2. Sequential stages of single terminal budding of yeastlike endosymbiotes of fermale *S. furcifera* Magnification, 2000 x (oil immersion).

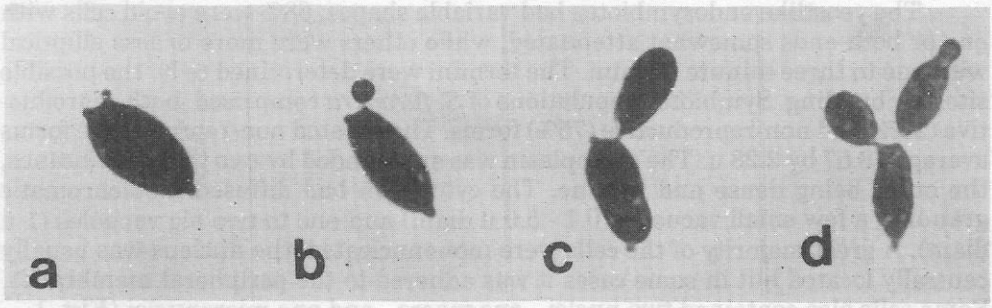

Fig. 3. Sequential stages of double budding of yeastlike endosymbiotes of female *S. furcifera*. Magnification, 2000 x (oil immersion).

Houk and Griffiths (1980) reviewed the host control of intracellular endosymbiotes of Homoptera, the transovarial infection, and their role in host nutrition. Endosymbiotes seem to fulfill the sterol requirements of planthoppers and also evidently produce antibiotic defense substances; the insect body becomes covered with mold if endosymbiotes are killed by heat treatment (Fredenhagen et al. 1987). Recently, Raguraman et al. (1988) reported that *N. lugens* nymphs reared on rice plants sprayed with derivatives of neem, *Azadirachta indica* A. Juss, seed had significantly less endosymbiotes and suffered frequent impairments than nymphs reared on untreated rice plants. Disruption of symbiotic associations between insects and microorganisms may lead to a novel pest control approach.

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