SHORT COMMUNICATIONS

Studies on the Endocellular Procaryotes in *Lohita grandis* (GRAY) (Hemiptera : Pyrrhocoridae) II. Cultivation of Presumptive Endocellular Procaryotes¹

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(Received August 26, 1980)

The presence of procaryotes in the cytoplasm of mycetocytes of insects is an accomplished fact. Such a microbial endosymbiosis is extremely wide spread among different groups of insects (BUCHNER, 1965). The endocellular procaryotes may be bacteria, fungi (yeast like), rickettsia like organisms or actinomycetes. These are usually located in the different parts of the insect body, eg., gut epithelium, fat body, haemolymph, malpighian tubules, gonads etc. When the insect

harbours only a single type of endocellular procaryote, it is known as monosymbiotic host, whereas pleurisymbiotic host contains two or more types which remain compartmentalised in the particular section of the mycetome.

In case of the insects belonging to the family Pyrrhocoridae, the procaryote-insect relationship has not been fully studied as very few attempts have been made so far to cultivate these procaryotes. KHAN (1974) made an attempt to isolate the procaryotes, culture them in the laboratories and reported the presence of a bacterium, *Alcaligenes faecalis* in *Dysdercus cingulatus* F. (Pyrrhocoridae : Hemiptera).

In *Lohita grandis*, the last section of the midgut is transformed to mycetome and is characterised by the presence of many parallel caecum like structures. The following is an attempt to show how the endocellular procaryotes found in *L. grandis*, are cultured in the laboratories.

The insect material was anaesthetized with Chloroform and sterilized externally in 0.1% Mercuric Chloride solution for 15 to 20 minutes. Then it was subjected to thorough washing with sterilized water. The insect was then dissected under a dissecting binocular microscope in aseptic condition, (in an ultra-violet ray sterilized in-

Table 1. CULTURAL CHARACTERISTICS OF ISOLATED ENDOCELLULAR PROCARYOTES
OF *Lohita grandis*

Tests	Endocellular procaryote	Minidentification of <i>Lactobacillus</i> (COWAN and STEEL, 1965)
1. Shape	Small rods*	Rods
2. Gram Staining	Positive	Gram positive in young cultures
3. Acid-Fast Staining	Non Acid-Fast	Non Acid-Fast
4. Motility Test	Motile	Typically non-motile
5. Spore	Non-sporing	Non-sporing
6. Carbohydrate breakdown		Attack sugars fermentatively
Glucose	Acid produced	
Sucrose	Negative	
Lactose	Acid produced	
Mannitol	Acid produced	
Glycerol	Negative	
7. Catalase Test	Negative	Negative
8. Methyl Red Test	Positive	
9. Voges-Proskauer Test	Negative	
10. Citrate Test	Negative	

* It forms chain within the cells of the mycetomal caecum (Fig. 1).

¹ Appl. Ent. Zool. **16** (2) : 162-164 (1981).

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Fig. 1. Photomicrograph of a smear of mycetomal caecum showing a chain of symbiotic bacteria (arrow) in a Phase-Contrast microscopic preparation. (Magnification $\times 675$)

Fig. 2. Photomicrograph showing a smear of cultured mycetomal bacteria. (Magnification $\times 1000$)

oculation room) to remove the mycetomal tissue. The mycetomal organ thus obtained was washed 3 to 4 times with sterilized Ringer's solution or distilled water. The last washing was used as an inoculum to ensure the aseptic nature of the washing material. The mycetomal tissue was then crushed in a drop of sterile Ringer's solution or distilled water to make a suspension. A loopful of this suspension was used to inoculate tubes of sterile Nutrient broth (composition: Beef extract-

10 gm, Peptone-10 gm, Sodium Chloride-5 gm, Distilled water-1000 ml; adjusted to pH 7.2-7.4). These tubes were then incubated at 30°C for 72 hours. After repeated trials turbidity was noticed indicating growth of the microorganisms. Pure culture of the microorganisms was obtained by following "Streak dilution plate technique". Prominent and clearly separated colonies were chosen from the streak dilution plate to streak Nutrient agar slants which after incubating for

48 hours at 30°C, were stored in a refrigerator for further use.

Different cultural and biochemical tests (COWAN and STEEL, 1965) were performed to identify the procaryotes. The identification was done according to the Bergey's Manual of Determinative Bacteriology (BREED, MURRAY and SMITH, 1957).

The following table gives an account of characteristics of the endocellular procaryotes of *Lohita grandis* (GRAY) and their comparison with the minidentification of *Lactobacillus* (COWAN and STEEL, 1965).

From the above mentioned characteristics it can be concluded that the endocellular procaryote of *Lohita grandis* (GRAY) is an organism very close to *Lactobacillus*.

Authors are grateful to Dr. J. MITSUHASHI, Division of Entomology, National Institute of Agricultural Sciences, Japan, for his help and encouragement.

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Courtship Behavior of the Melon Fly, *Dacus cucurbitae* COQUILLET (Diptera : Tephritidae)¹

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(Received November 7, 1980)

BACK and PEMBERTON (1917) gave a brief description of the mating behavior of the melon fly, *Dacus cucurbitae*, which occurs at dusk. KOBAYASHI et al. (1978) described certain aspects of the courtship behavior of this species, using mass-reared individuals, and demonstrated that the gland complex of the rectal sac of the males, suspected as the source of a pheromone, was attractive to virgin females. The presence of male pheromone in the melon fly is also suggested by SUGIMOTO (1979), who observed smoke emission at dusk by sexually mature flies confined densely in a cage. Through these works, it now

appears that the courtship behavior of the melon fly includes stridulation (wing vibration producing a buzzing sound) and pheromone release by males, as in the case of many other tephritid flies. However, to date, the full sequence of the mating behavior of the melon fly is still unknown. This paper presents a detailed description of the courtship behavior of melon flies under laboratory conditions, including the response of antenna-excised flies to conspecifics.

Two strains of the melon fly were used, one the laboratory (L) strain reared on an artificial diet through about 49 generations at the Yaeyama Branch of the Okinawa Agricultural Experiment Station, and the other the wild (W) strain obtained as larvae from infested bitter cucumber, *Momordica charantia* var. *pevel*, collected in Okinawa, in June, 1979. Adult flies were sexed within a day after emergence and reared until sexual maturity in screen cages (30×30×45 cm) on water, sugar, protein hydrolysate, and pieces of squash, *Cucurbita moschata*, at 27.5±1.5°C with a 15-h photophase. Observations of the courtship behavior were made under semi-natural temperature and light conditions and were tape-recorded. A single pair of virgin flies was introduced into the screen cage at 17:00 about 2 hr before sundown. Because sexual maturation is delayed in the W-strain compared with the L-strain, observations were

¹ Appl. Ent.Zool. **16** (2) : 164-166 (1981).