

Indigenous microflora of the West Indies fruit fly, *Anastrepha obliqua* (Diptera : Tephritidae).

Teresita MURILLO, Patricia RIVERA, F. HERNANDEZ and L.F. JIRON

INDIGENOUS MICROFLORA OF THE WEST INDIES FRUIT FLY, *ANASTREPHA OBLIQUA* (DIPTERA : TEPHRITIDAE).

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ABSTRACT - Bacteriological and ultrastructural studies of recently and 24 hours post oviposited eggs of *Anastrepha obliqua* (Diptera ; Tephritidae) were carried out. Also, bacteriological cultures from intestine of this fly were done. The specimens were inoculated on blood agar and Mc Conkey culture media. *Klebsiella oxytoca*, *K. pneumoniae*, *Enterobacter agglomerans*, and *E. cloacae* were the most common bacteria isolated from the tested samples. We found that the indigenous microflora of *A. obliqua* is similar to previously reported microflora for other species belonging to Tephritidae.

The bacterial flora of fruit flies often includes *Klebsiella oxytoca*, *Enterobacter agglomerans* (*Erwinia herbicola*), and *Enterobacter cloacae* (all Enterobacteriaceae), which have been called «Fruit Fly Type Bacteria» (Drew and Lloyd, 1989).

These bacteria benefit the host by providing several biochemical functions, including protein and pectin hydrolysis, threonine and methionine synthesis, fruit detoxification and protection against pathogenic bacteria (Howard *et al.*, 1985 ; Courtice and Drew, 1984). Moreover, by adding these bacteria to the artificial diet of *Dacus* fruit flies, both oviposition and hatching rates are increased (Drew *et al.*, 1983). This relationship has been interpreted as symbiosis by some authors (Girolami, 1983 ; Manousis

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RESUME - Etudes bactériologiques et ultrastructurales d'oeufs récemment pondus de la mouche des fruits *Anastrepha obliqua* (Diptera ; Tephritidae). Cultures bactériennes à partir de l'intestin de cet insecte. Bactéries isolées de ces échantillons : *Klebsiella oxytoca*, *K. pneumoniae*, *Enterobacter agglomerans* et *E. cloacae*. La microflora répertoriée est similaire à celle rapportée sur les autres espèces du genre.

and Ellar, 1988), but others argued that these bacteria have not been demonstrated to be essential for the survival of the fly (Drew and Lloyd, 1989). The aim of this paper is to define the microflora associated with *Anastrepha obliqua* (Macquart), an important fruit fly pest in tropical America (Jirón and Hedström, 1988).

MATERIALS AND METHODS

The fruit fly colonies (F 3) were maintained in one cubic meter cages, at a mean temperature of 21.5°C at the «Organismo Internacional Regional de Sanidad Agropecuaria» (OIRSA), San José, Costa Rica.

Egg flora : Sterile agar hemispheres (5 cm in diameter) covered with a layer of a mixture of beeswax and paraffin were placed in each cage to collect eggs. Within half-a-minute after oviposition, eggs were aseptically extracted from the agar hemispheres and inoculated on bacterial culture media : blood agar (Trypticase soy base BBL) and McConkey.

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Intestinal flora : Fifteen adult flies were externally disinfected by immersion in 70% ethanol for 2 minutes and subsequently washed with distilled water. Then, the intestine was aseptically extracted and inoculated on the bacteriological culture media.

Cage flora - Two blood agar plates were placed on the cage floor for two hours and then incubated.

All the inoculated culture plates were incubated at 37°C for 24 hours. The colonies were analysed by the Gram staining, oxidase reaction and identified using the automatic Quantum II micromethod (Abbot Lab.).

Ultrastructure - Two 20-egg groups were fixed in 2.5 % glutaraldehyde, post fixed in 1% osmium tetroxide, dehy-

drated, critical point dried, and analyzed under scanning electron microscopy (Hitachi S-570). In one, the eggs were immediately extracted from the agar hemispheres after oviposition and the other, was extracted 24 hours later.

RESULTS AND DISCUSSION

Table 1 shows the bacterial species isolated from eggs as well as from the intestine ; most common in both were *Enterobacter agglomerans*, *E. cloacae*, *Klebsiella oxytoca*, and *K. pneumoniae*.

Acinetobacter lwoffii, *Enterobacter gergoviae* and *Proteus* sp. were isolated almost exclusively from the intestine. They are components of the intestinal flora, not necessarily transmitted throughout eggs and could be obtained by the adult during its exploratory behavior in both

TABLE 1 - Eggs and intestine microflora of *Anastrepha obliqua* (Diptera ; Tephritidae).

| Bacteria | N° strains isolated | |
|---------------------------------|---------------------|----------------------|
| | Eggs (n = 17) | Intestine (n = 15) * |
| <i>Acinetobacter lwoffii</i> | 1 | 4 |
| <i>Enterobacter agglomerans</i> | 5 | 4 |
| <i>Enterobacter cloacae</i> | 3 | 3 |
| <i>Enterobacter gergoviae</i> | 0 | 4 |
| <i>Klebsiella oxytoca</i> | 2 | 4 |
| <i>Klebsiella pneumoniae</i> | 2 | 4 |
| <i>Proteus mirabilis</i> | 0 | 1 |
| <i>Proteus vulgaris</i> | 0 | 1 |
| <i>Proteus ratgeri</i> | 0 | 1 |
| <i>Pseudomonas fluorescens</i> | 0 | 1 |
| <i>Pseudomonas cepacea</i> | 0 | 1 |
| Negative samples | 6 | 0 |

* - 4 strains, Gram negative bacilli unidentified.

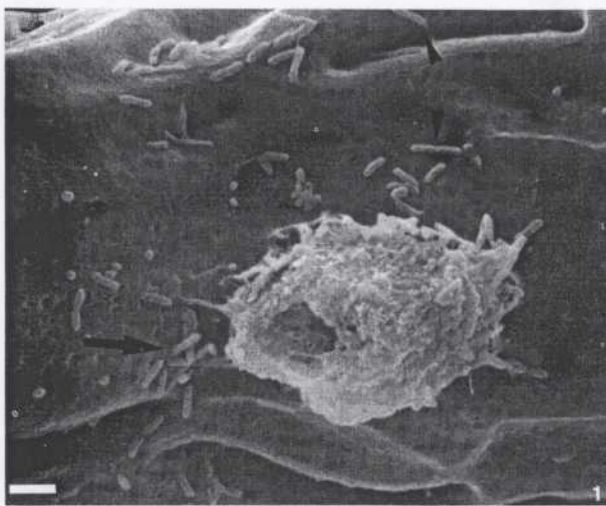


Figure 1 - SEM micrograph of an egg processed 24 hours after oviposition. Some bacteria (arrow) are localized near the micropyle, the arrow heads show bacilli in their division process (Barr = 3 μ m).

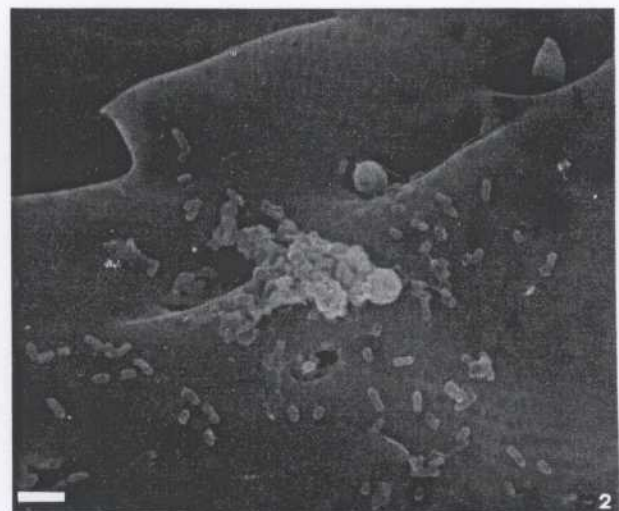


Figure 2 - SEM micrograph of an ovipositor shows different shaped and size bacteria (Barr = 3 μ m).

mature fruits and leaf surfaces, or while feeding on bird drops (Prokopy and Roitberg, 1984). From the cage a strain of *Yersinia enterocolitica* was isolated; this bacterium is involved in cases of human diarrhea, which suggests that fruit flies might be involved in the transmission of some human pathogenic bacteria.

Micrographs of recently oviposited eggs show some bacterial deposits mostly on the respiratory horn region and at the opposite end of the egg. This suggests that the egg is colonized by rod bacteria, at least just before oviposition. In eggs processed 24 hours after oviposition, bacteria were

on the whole egg surface, and it is possible to observe rod-shaped bacteria in their division process (Fig. 1). We also observed bacilli attached to the surface of ovipositors (Fig. 2), which suggests that the eggs could become colonized while traversing the oviposition canal.

In conclusion, *A. obliqua* is colonized by the same genera of Enterobacteriaceae reported as Fruit Fly Type Bacteria in other tephritid flies (Drew and Lloyd, 1989), that we call «indigenous microflora» in order to point out their mutual advantages, instead of the term «symbiosis».

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RESUMEN - Estudios bacteriológicos y ultraestructurales de huevos recientemente puestos de la mosca de los frutos *Anastrepha obliqua* (Diptera : Tephritidae). Cultivos bacterianos a partir del intestino de este insecto. Bacterias aisladas de estos muestreos : *Klebsiella oxytoca*, *K. pneumoniae*, *Enterobacter agglomerans* y *E. cloacae*. La microflora repertoriada es similar a la que se ha dado para las otras especies del género.