Research Note

BACTERIA ASSOCIATED WITH THE WEST INDIAN FRUIT FLY ANASTREPHA OBLIQUA *(MACQUART) (DIPTERA: TEPHRITIDAE)*[1]

Current quarantine research with the West Indian fruit fly, *Anastrepha obliqua*, by the University of Puerto Rico-Agricultural Experiment Station has required mass rearing of the insect for artificial infestation of mango fruit. Initially, bacterial contamination of larval-rearing media was a persistent fruit fly breeding problem. Since this situation has been previously reported about other members of this genus,[2, 3] we have isolated and characterized the most common bacterial contaminants encountered while rearing the larvae.

Bacterial contaminants were isolated and purified from rearing media with standard microbiological methods. Plate-count agar was used as culture medium and incubated at 35° C. Gram-negative bacteria from pure colonies were subsequently characterized with the API 20E system to identify Enterobacteraceae and other gram-negative bacteria (API Diagnostics, Div. Sherwood Medical, 200 Express St., Plainview, New York 11803).

Most prevalent bacteria isolated were *Enterobacter cloacae*, followed by *Pseudomonas* sp. and *Serratia marcescens*. *Enterobacter cloacae* and *Pseudomonas* sp. have been isolated from *A. suspensa* and its parasitoid *Biosteres longicaudatus*.[4] We observed that larval medium pH and rearing room temperature greatly influenced bacterial growth. Hydrochloric acid (1 M) concentrations of 15 ml per liter of papaya-agar diet (pH 5.6) allowed unrestricted bacterial growth, whereas 30 ml and 40 ml HCl per liter (pH 4.9 and 4.4, respectively) reduced or inhibited bacterial growth. Analogously, little bacterial development was observed at temperatures at or below 27° C. Temperatures at or above 30° C largely worsened bacterial contamination problems. High larval mortality was often associated with enhanced bacterial growth under high temperature conditions (i.e., 30° C). Similar conditions have been described by Greany et al. in Florida[4] for the close ally *A. suspensa*.

Internal location of bacteria in adult flies was determined after surface sterilization of the entire body in 0.15% NaOCl for 2 minutes. We dissected laboratory-reared and field-collected fruit flies and removed their reproductive and digestive systems. Organs were surface-sterilized as described and placed on nutrient agar plates. *Enterobacter cloacae*, *S. marcescens* and *Pseudomonas* sp. were isolated from male and female digestive tracts. *Pseudomonas* sp. was the only bacteria isolated from testicles and ovaries. Bacteria appear to be transmitted to offspring through surface contamination of eggs from ovarial and proctodaeal

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[2]Mallory Boush, G., S. M. Saleh and R. M. Baranowski, 1972. Bacteria associated with the Caribbean fruit fly. *Environ. Entomol.* 1: 30–33.

[3]Rubio, R. E. P. and M. W. McFadden, 1966. Isolation and identification of bacteria in the digestive tract of the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 59: 1015–016.

[4]Greany, P. D., G. E. Allen, J. C. Webb, J. L. Sharp and D. L. Chambers, 1977. Stress-induced septicemia as an impediment to laboratory rearing of the fruit fly parasitoid *Biosteres* (*Opius*) *longicaudatus* (Hymenoptera: Braconidae) and the Caribbean fruit fly *Anastrepha suspensa* (Diptera: Tephritidae). *J. Invert. Pathol.* 29: 153–61.

sources. Bacterial colonies failed to grow on 0.15% NaOCl surface sterilized eggs. Sodium benzoate (0.03%) solutions routinely used in rearing procedures did not seem to affect bacterial growth. Most researchers recommend optimization of larval culture conditions as a preferred measure of bacterial control.[4, 5] Cultural improvements in rearing techniques of *A. obliqua* in our investigation have included proper sanitation, regular sterilization of cages, keeping larval diet pH below 4.9, avoidance of adult and larval crowding and temperature control at 27° ± 2° C. These measures have efficiently controlled most of the bacterial contamination problems encountered in our colonies.

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[5]Kamasaki, H., 1970. Some pathogens and pests associated with tephritid flies in the laboratory. *J. Econ. Entomol.* 63: 1353–355.