LeFever, H.M. and R.C. Moore. Kansas State Teachers College, Emporia.
Characterization of a micro-organism in Drosophila medium.

In many laboratories the control of bacterial and fungal contamination in Drosophila food medium has been of concern for many years (Felix 1969). Some stock cultures in various laboratories throughout the country have been infected with bacterial growth which gradually spreads to vials and in some instances to all viable cultures.

The objectives of the present study were to bring about isolation, culture, identification and ultrastructural examination of the organism in question.

Isolation and culture: A sample of the organism in question was obtained from contaminated Drosophila media and a streak for isolation was carried out on nutrient agar plates. Following inoculation of the plates there was a 24 hr period of incubation at 37°C. After this period, observations were recorded and inoculation of metabolic media was carried out.

Antibiotic sensitivity: Sensitivity tests were carried out using petri plates containing a blood agar base of 5.0% human blood. Antibiotics used were the following: chloromycetin, neomycin, erythromycin, kanamycin, novobiocin, penicillin, streptomycin and tetramycin. The diameter of the zone of inhibition around each sensitivity disc was measured and recorded.

Growth: Observation of growth was carried out on triple sugar iron agar and nutrient ag agar slants; recordings were taken at 4.0 hrs, 6.0 hrs, 8.0 hrs, 10.0 hrs, 24.0 hrs and 48.0 hrs.

Staining: A gram, spore, and capsule stain were carried out using standard laboratory methods. A gram stain was procured at each recording taken during the period of growth observation.

Electron microscopy: Fixation and embedding were carried out using a standard procedure developed by Kellenberger, Ryter and Sechaud (1958). Photomicrographs were taken on a Hitachi model HS8 electron microscope.

Metabolic tests employed in this study are listed in Table 1 with their results.

Table 1. Results of metabolic tests read at 48 hrs.

Triple sugar iron agar	no gas, no H_2S, alkaline slant, acid butt	Triple sugar iron agar	no gas, no H_2S, alkaline slant, acid butt
Nutrient broth	positive	Lactose	negative
Nutrient agar	positive	Mannitol	slight positive
Blood agar	positive	Urea	negative
Citrate	negative	Gelatin	negative
Motility	positive	Nitrates	negative
Indol	negative	Lysine decarboxylase	slight positive
Salicin	negative	Methyl red	negative
Sucrose	slight positive	Vogues-proskauer	negative
Dextrose	positive, no gas	Catalase	positive

When inoculated upon solid media this organism has a mucoid growth with separate colonies being slightly raised with smooth margins after four hours incubation.

After six hours, growth is palisade and motility is apparent at this stage.

From eight to ten hours, the organism appears pink to red in color with the palisade arrangement not as pronounced; the pigmentation taking on a red-brown appearance after twelve hours. A crust-like covering may be present at this time, thus making the colonies appear dry and folded.

Gram stains taken at 1.0 hr, 2.0 hrs, 6.0 hrs, 10.0 hrs and 24.0 hrs indicated a paucity of gram positive rods with the presence of coccoid forms. Upon using the same technique of examination at 48.0 hrs the organism appeared gram negative. To rule out the possibility of contamination further isolation procedures were carried out, but the results remained the same.

Table 2
Antibiotic sensitivity determined with respect to diameter of zones of inhibition

	Concentrations		
	High	Medium	Low
Chloromycetin	2.3 cm	2.0 cm	1.8 cm
Neomycin	1.8	1.5	1.5
Erythromycin	2.5	2.3	2.0
Kanamycin	2.0	1.7	1.5
Novobiocin	2.0	1.7	1.5
Penicillin	2.3	2.6	2.0
Streptomycin	1.0	1.0	0.0
Tetramycin	1.9	1.5	1.3

The capsule and spore stains from a 24.0 hr culture were negative.

Electron microscopy revealed a thick cell wall encasing the organism in all preparations examined; no specialized intracellular structures could be distinguished in any of the preparations.

The microorganism isolated and examined in this study suddenly appeared in culture media of Drosophila melanogaster; its presence seemed to overwhelm the Dipteran population thereby bringing about total loss of the cultures.

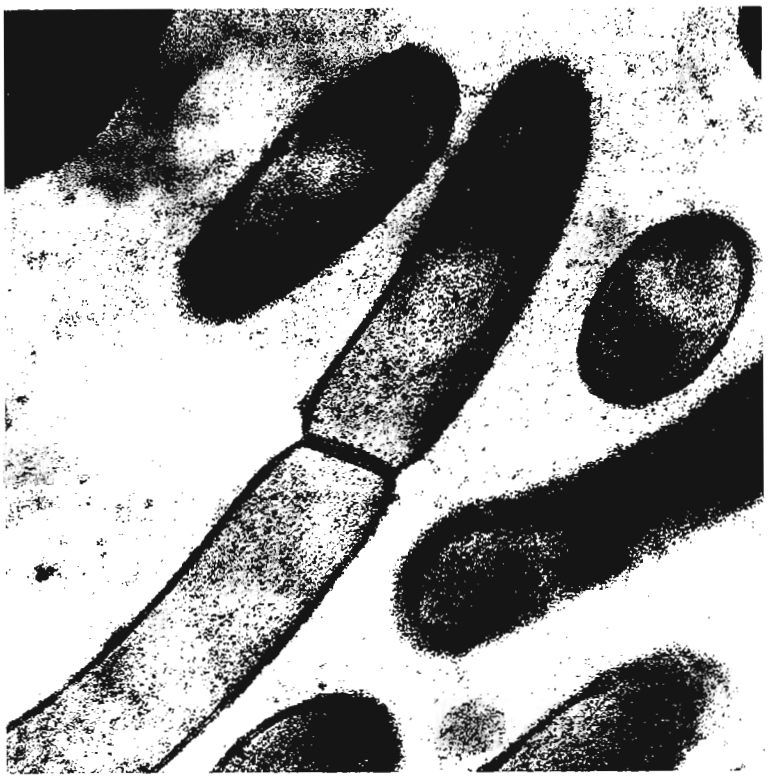

Figure 1. Electron micrograph of bacteria contaminating Drosophila culture media. 48,140 X.

Contamination involving this particular organism is difficult to control in spite of standard sterilization procedures. The organism's resistance to control methods is probably due to the thick cell wall as seen by electron microscopy (Figure 1).

To bring about control of this organism a 50.0% solution of a tincture of Zephiran (Winthrop Laboratories) was used in treating all contaminated media and containers, after which the organism was no longer detectable. Most soaps and alcohol solutions below 80.0% were not effective in controlling the organism; in fact it seemed to thrive in some cases. Antibiotic sensitivity tests indicate a susceptibility to chloromycetin, erythromycin, and penicillin (Table 2).

References: Felix, R. 1969, DIS 44:131; Kellenberger, E., A. Ryter and J. Sechaud 1958 J. Biochem. and Biochem. Cytol.

Gassparian, S. University of Isfahan, Isfahan, Iran. Reproduction of D. melanogaster for different cross breedings.

For the production of virgin females of D. melanogaster in some laboratories, it is customary to separate and transfer the female from the culture medium when the pupa is nine days old, a few hours before the opening of the wings. As this method is time consuming, a new and more satisfactory method has been developed in the Genetics Laboratory at the University of Isfahan. On the seventh day when the pigments of the eye are formed, the pupae are transferred to several new, small, sterile glass containers which have sufficient nutrients; those pupae which were on the glass walls of the original container are moved by a special spatula, and whose which were on cotton wool being transferred by forcepts with the cotton wool, in order to avoid direct contact. Two days after this procedure the virgin females and males are recognizable and thus separated. By this method the time involved is less than the traditional one and the casualty rate is about 10%.