AEROBIC BACTERIA IN THE MIDGUT OF *SIMULIUM DAMNOSUM* LARVAE [1]

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Bacteria are often omitted from descriptions of blackfly midgut contents because they may be mistaken for inorganic particles, or are not considered to be food organisms, or because bacteriological procedures are necessary for their identification. Certain investigators have recognized their value in nourishing blackfly larvae. Fredeen (1960, 1964) found that *Simulium venustum* Say, *S. verecundum* Stone & Jamnback, and *S. vittatum* Zetterstedt developed to adults, and *S. arcticum* Malloch to the last larval stage, when fed only on washed suspensions of *Bacillus subtilis, Aerobacter aerogenes,* or *Escherichia coli.* There was little difference in larval growth when using live versus ultraviolet-irradiated *A. aerogenes* as food, indicating that dead bacteria also had food value. It was suggested that soil and sewage bacteria contributed to *S. arcticum* outbreaks. Fredeen (1963) stated that most bacteria in streams are dead; if there are 100,000 to 25,000,000 bacterial cells per ml of water, only up to 25,000 would be viable. Others who reported blackfly larvae thriving on bacteria were Forbes (1912), Emery (1914), Jobbins-Pomeroy (1916), Wilhelmi (1920), Petersen (1924), Snow *et al.* (1958), and Carlsson (1967). *S. argyreatum* (Mg.) Lundström (= *S. erythrocephalum* de Geer) and *S. equinum* L. (= *Wilhelmia equina* L.) often occur in polluted water. Snoddy and Chipley (1971) isolated from the intestines of larvae of *S. underhilli* Stone & Snoddy the bacteria *Enterobacter cloacae, Pseudomonas aeruginosa, Aeromonas hydrophila, Micrococcus* sp., and *E. coli.* They suggested that the material filtered by the larvae could be a valuable indicator for consistent evaluation of water pollution.

Knaysi (1951) stated that water constitutes 80–90 percent of the vegetative bacterial cell. Its minerals include phosphorus, magnesium, and sulfur. The dry matter consists of proteins (50–80 percent nucleoprotein), 10–30 percent carbohydrates, and up to 40 percent lipids.

PRESENT STUDY

While investigating the particulate matter ingested by *S. damnosum* Theobald larvae, a determination was made of aerobic bacterial types and numbers in the midguts of larvae collected from the concrete spillway of the agricultural dam at Soye, near Bolgatanga in the Upper Region of Ghana. A limited study was also made on fungal types and numbers. This spillway, with water pH 6.0, was described by Burton & McRae (1965).

DETERMINATION OF AEROBIC BACTERIAL TYPES. a. Fifteen mature larvae were placed into an antiseptic, 1 percent Savlon [5] for 5 minutes, then were rinsed repeatedly in excess normal saline. The midgut contents of each larva, bound by the peritrophic membrane, were dissected out and placed into individual test tubes containing Todd Hewitt broth (Todd & Hewitt, 1932), then macerated with a sterile needle. After incubation at room temperature (25° C) for 48 hours, each tube was subcultured to nutrient agar plates. A special study was made on broth culture #1, which was subcultured to 2 plates each of nutrient agar, brain heart infusion agar, and Sabouraud-like dextrose agar. One each of these pairs of plates was incubated at 25° C and the other at 37° C, but the latter temperature did not provide any new colony types. No anaerobic organisms were determined, since thioglycollate medium was not available at the time of this study. Results from these cultures represented only rapidly-growing bacteria, which would overgrow slow-growing organisms, and their metabolic products probably changed the broth medium to a pH value unfavorable for certain other bacteria. After overnight incubation, colony types were inoculated again into Todd Hewitt broth from each of the agar plates, and resulting subcultures were checked for bacterial motility and Gram staining characteristics. Additional subcultures were made to nutrient agar to confirm pure cultures and to check for colony morphology and pigmentation.

b. Five additional larval midguts were removed aseptically, ground in a tissue grinder with 10 cc of normal saline, and after serial dilutions were plated on nutrient agar and Sabouraud's dextrose agar. Studies were made on family types and other characteristics.

c. Finally, fresh slides were prepared of the midguts of 15 other mature larvae. Following motility studies the slides were air-dried for 48 hours, then were stained by Gram's method. Thus the midguts of 35 mature larvae were utilized in this initial study.

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[5] Savlon, I.C.I., is chlorhexidine gluconate, 1.5% up; and cetrimide B.P. 15% w/v, as a concentrate for hospital use.

RESULTS. All 20 midguts in a plus b (100 percent) had Azotobacteraceae species, 7 (35.0 percent) had Bacillaceae, 1 (5.0 percent) had Micrococcaceae, and 1 (5.0 percent) had Pseudomonadaceae. Three of the 5 midguts in study b had *Penicillium,* and 3 had *Aspergillus,* one midgut having both. As regards the 15 midguts in study c, the bacterial forms, motility, and Gram-staining results agreed fairly well with those obtained in studies a and b. Many unicellular green algae and diatoms were also seen in these midguts, as well as some crustacean appendages and insect fragments.

The Azotobacteraceae occurred as short rods, cocco-bacilli, or coccus- or yeast-like forms which grew well in nitrogen-free media. There were both Gram-negative and Gram-positive forms, confirming Norris and Chapman (1968) concerning Gram-variability of Azotobacteraceae. Almost all were motile. The non-motile coccobacillus forms producing mucoid white to buff colonies conformed to descriptions of *Azotobacter beijerinckii* (Lipman). The peritrichously flagellate, coccoid, almost spherical forms which produced greyish-white mucoid or translucent colonies were identified as *Azotobacter agilis* Beijerinck. The rods that produced buff to tan mucoid colonies and microcysts were identified as *Azotobacter chroococcum* Beijerinck. Identifications were based on descriptions in Breed *et al.* (1957), Skerman (1967) and Norris and Chapman (1968).

The azotobacters occur both in soil and water. They fix atmospheric nitrogen, and can consume as much as 100–150 μg N/gm of carbohydrate in 24 hours; they have the highest rate of oxidation or respiratory rate of any known cells, the Q_{O_2} values being as high as 4,000–5,000 (Werkman and Wilson, 1951). The carbohydrates and other sources of carbon are transformed by them to CO_2, H_2O and cell substance. They deposit lipoid inclusions in the cytoplasm. Their growth is controlled by available phosphorus, and they generally cannot develop in soil having a pH less than 6.0 (Waksman, 1952).

The Bacillaceae (genus *Bacillus*) isolated were Gram-positive, motile rods which produced spores, and yielded dry white and mucoid tan or buff colonies. The single isolate of the Pseudomonadaceae was a Gram-negative, motile rod producing a mucoid, blue-green colony. The isolate of Micrococcaceae was a Gram-variable, mostly Gram-negative, non-motile form which occurred singly, in pairs, in tetrads, and formed a yellow, spherical colony.

DETERMINATION OF BACTERIAL AND FUNGAL COUNTS IN MIDGUTS. Ten *S. damnosum* mature larvae were placed into 1 percent Savlon for 5 minutes, and rinsed in excess normal saline. Each midgut was removed aseptically, and ground in a sterile tissue-grinder with 10 ml of normal saline. Further 10-fold dilutions were made, and 0.1 ml of the final sample was spread on nutrient agar and Sabouraud's dextrose agar plates.

RESULTS. Bacterial colony numbers from the 10 midguts after 2 days incubation at 25° C were: 1,400,000; 240,000; 89,000; 1,100,000; 1,200,000; 280,000; 4,000,000; 500,000; 90,000; and 200,000. The respective numbers of fungal colonies were: 7,000; 1,000; 1,000; 1,000; 6,000; 7,000; 2,000; 7,000; 1,000; and 2,000. Each viable unit resulting in a colony may have originated from either one organism or from a group of organisms (Collins, 1967).

SUMMARY. The midgut contents of 20 *Simulium damnosum* Theobald mature larvae taken from an agricultural dam spillway near Bolgatanga, Ghana, were cultured utilizing simple media, to determine aerobic bacterial types and numbers. All 20 (100 percent) of the midguts had Azotobacteraceae species, 7 (35 percent) had Bacillaceae, 1 (5 percent) had Micrococcaceae, and 1 (5 percent) had Pseudomonadaceae. In 10 additional midguts, aerobic bacterial colonies ranged from 89,000 to 4,000,000 per midgut, and fungal constituents from 1,000 to 7,000. Twenty-five degrees C was found to be superior to 37 degrees C for bacterial growth and replication.

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BOOK REVIEW

VENEZUELAN ENCEPHALITIS. PROCEEDINGS OF THE WORKSHOP-SYMPOSIUM ON VENEZUELAN ENCEPHALITIS VIRUS. WASHINGTON, D.C., 14–17 SEPTEMBER 1971. Scientific Publication No. 243. Pan American Health Organization, 525 Twenty-Third Street, N.W., Washington, D.C., 416 pages. $5.00.

Venezuelan equine encephalitis (VEE) has emerged as the most important mosquito-borne disease in the Western Hemisphere in recent years. The epidemic of 1971 in Texas, the continued endemic presence of the virus in Florida and repetitive extensive outbreaks in horses and man from northern South America into Mexico document the importance of VEE. All workers in mosquito control and mosquito-borne diseases who have need for a knowledge of VEE recognize the need for a summary of the research and literature on this disease.

The Pan American Health Organization, in recognition of the above needs, held a four-day workshop-symposium on VEE and invited 130 scientists and administrators to attend. This publication represents the papers and discussion from that meeting. The contents are divided into eight sections that encompass the history of VEE, antigenic characteristics of the virus, host-virus interaction, epidemic and endemic behavor, a review of the Mexico-Texas outbreak, avian and vector hosts and prevention and control.

Readers of this journal will find this is a definitive reference source to guide them in the event of further epidemics and a ready source of authoritative information. There is adequate consideration of the range of mosquito vectors that become involved in the epidemic and endemic cycles and how they interact with the range of vertebrate hosts and virus strains. The alternative approaches to control, vector abatement and vaccination, are placed in proper perspectives.

The table of contents is detailed, but there is no index, and this means that the reader may have to peruse a number of sections to assure he has a complete understanding of any aspect of the problem. As in all proceedings, there are irregularities in presentations and continuity of considations is not complete. However, the publication is well edited.

This review is highly recommended as the most definitive summary of knowledge and references that is available on VEE, and the Pan American Health Organization is commended for its foresight in organizing the meeting and making the 416-page proceedings available in such a short period.

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