Microbial Ecology of the Gut in Laboratory Stocks of the Migratory Grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae)

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Mean pH values in pooled samples of foregut, midgut, and hindgut from adult *Melanoplus sanguinipes*, which had been raised in the laboratory on barley shoots and wheat bran, were 5.15, 6.39, and 5.98, respectively. Homogenates of midgut/hindgut sections and frass (feces) yielded colony counts of bacteria by the spread plate method of 5.7 to 5.9 and 5.3 to 5.5 log_{10} colonies per mg, respectively; there were no significant differences (P > 0.05) between counts obtained on several media or on media incubated aerobically or anaerobically. There was no evidence of significant populations of protozoa, fungi, or obligately anaerobic bacteria associated with the gut. A total of 168 pure strains of bacteria isolated from the gut sections were characterized and assigned to 11 taxonomic groups, including *Enterococcus* spp., *Serratia liquefaciens*, *Pseudomonas* spp., and *Enterobacter* spp. Numbers of *Enterococcus* spp. in the gut were 2 to 3 orders of magnitude higher than those of the other genera. Strains representing only four of the groups were recovered from frass fed to the grasshoppers; the barley shoots, which were raised in sterile soil, appeared virtually sterile. Examination of the gut wall by scanning electron microscopy revealed the presence of epimural bacteria in the foregut and hindgut but not in the midgut. The distribution of epimural cocci and bacilli differed with the gut section examined. Numerous spherical to ovoid structures up to 10 μm in diameter, which were not identified, were associated with the microvillus surface of the midgut epithelium. Acetate was present in gut, hemolymph, and frass, and it was shown that representative isolates of *Enterococcus* spp. and *Enterobacter agglomerans* produced acetate when incubated in an aqueous suspension of bran. The egestion time of solid digesta, as measured with methylene blue-stained barley shoots, was 3.0 to 5.7 h. The results show that *M. sanguinipes* supported extensive indigenous populations of luminal and epimural bacteria in the gut which were composed predominantly of facultatively anaerobic species; the relatively short egestion time, indicating rapid passage of digesta through the gut, was consistent with the microscopic appearance of digesta residues in frass and could account, at least in part, for the absence of a significant population of obligately anaerobic bacteria from the gut.

Members of the orthopteran family Acrididae cause significant damage to graminaceous crops in North America. In the limited number of species studied so far, the normal gut microflora is abundant and relatively simple, but little is known of the relationships which may exist between the gut flora and the physiology of the host insect.

In laboratory stocks of the desert locust, *Schistocerca gregaria*, the predominant flora of the midgut and hindgut regions of adult insects, in which primarily indigenous forms would be expected, was composed of members of the family *Enterobacteriaceae* and motile streptococci (37); in the foregut, a wider range of bacterial types, including aerobic and anaerobic sporeformers, was identified, but many of these were considered to be adventitious. Similar results were obtained by Hunt and Charnley (24) for laboratory-raised adult male *S. gregaria*; the numbers of members of the family *Enterobacteriaceae* isolated increased posteriorly from foregut to rectal sac, and *Enterobacter agglomerans* was the dominant organism. The highest densities of streptococci occurred in the hindgut, although numbers were 10 to 100-fold lower than those of members of the family *Enterobacteriaceae*. Because insects raised axenically appeared to develop and reproduce normally, Charnley et al. (10) concluded that the gut flora in this species did not contribute significantly to host nutrition.

Few studies of the gut flora of grasshoppers have been reported. Bucher and Stephens (5, 6) characterized 67 strains of bacteria isolated from wild grasshoppers collected in western Canada over a 5-year period. Whole insects were surface sterilized and triturated in sterile water; therefore, the isolates may be assumed to represent the gut flora. The predominant flora consisted of members of the family *Enterobacteriaceae*, *Brevibacterium* spp., and *Streptococcus* spp., with other aerobic and facultatively anaerobic genera present in smaller proportions. No attempt was made to enumerate the flora in individual insects. The dominant organism in the gut flora of the Australian acridid, *Caelidina captiva*, was *E. cloacae* (11), but there was no evidence of an interaction between the genotypes of this organism and the host based on either isoenzyme (11) or plasmid (12) variation.

In recent years the potential for biological control of insect pests, including grasshoppers, has attracted considerable interest as an ecologically and environmentally favorable alternative to the use of chemical pesticides (25). Since ingestion of the agent by the insect may in some cases represent the preferred route of infection, interactions between the pathogen and the gut flora of the host could influence the capacity of the agent to initiate or sustain pathogenesis. For example, production of antifungal metabolites by gut bacteria may help to protect the host against gut invasion by entomopathogenic fungi (17, 18). As a founda
tion for studies of such interactions, a more comprehensive knowledge of the microbial ecology of the gut in normal acidids is required. The objective of the work reported here was therefore to investigate the gut flora and its ecology in *Melanoplus sanguinipes*, the dominant grasshopper species in western Canada. During the work, rigorous anaerobic techniques were applied to determine whether the insects supported a significant population of obligately anaerobic bacteria in the gut.

**MATERIALS AND METHODS**

**Maintenance and dissection of grasshoppers.** A nondiapause strain of *M. sanguinipes* (27) was reared in the laboratory. Each instar was kept in a separate glass fish tank (30 to 35°C) equipped with a continuously illuminated 75-W incandescent light bulb. The insects were fed wheat bran and fresh shoots of 7-day-old barley (Hordeum vulgare var. Harrington) which had been raised in sterilized soil. Adult grasshoppers were provided with a container of wet sand for egg laying; this was subsequently removed to an empty tank to initiate a new developmental cycle. Between cycles, the tanks were rigorously cleaned and sterilized to avoid introduction of entomogenous contaminants.

**Sampling of grasshoppers.** Adult insects (35 to 40 days posthatching) were removed from the colony and placed on filter paper in individual petri plates. They were starved, usually for 4 h, and then anesthetized under CO₂ and surface sterilized by swabbing with tincture of iodine followed by 70% ethanol. The body cavity was opened aseptically and under a stream of O₂-free CO₂ to minimize exposure to air. Excess hemolymph was absorbed with sterile filter paper. The whole gut or gut sections, including contents, was ligated, freed of malpighian tubules, removed, and prepared as described below for microbiological analysis, scanning electron microscopy (SEM), or the determination of ecological parameters. The wet weight of gut and contents was determined by weighing the insect before and after dissection. When volatile fatty acids (VFA) in the gut fluid were to be determined, the gut section removed was immediately placed on a block of dry ice covered in aluminum foil and frozen prior to further processing. When hemolymph was required, an incision was made in the neck region and fluid was withdrawn by micropipette for immediate VFA analysis. Frass (fecal pellets) deposited during the period of starvation was collected aseptically, immediately weighed, and frozen at −70°C.

**Microbiological analysis.** To examine the gut microbiota, tissue obtained as described above was rapidly transferred under CO₂ to a tube containing 0.5 ml of anaerobic dilution solution (3) and homogenized manually at 22°C with a sterile Potter-Elvehjem tissue grinder. Whole gut sections were used in preference to gut contents alone so that both luminal and possible epimural (wall-adherent) components of the gut microbiota would be included. Frass was thawed and similarly homogenized. To examine the diet, fresh barley shoots (1 g) were cut, pooled, and homogenized in anaerobic dilution solution; bran (1 g) was suspended in 5 ml of sterile water and incubated at ambient temperature (20 to 22°C) for 24 h. The homogenates were examined microscopically in wet mounts and Gram-stained smears, and dilutions of the homogenates and bran suspensions were prepared in an anaerobic dilution solution. Samples (0.1 ml) of appropriate dilutions were then used to inoculate spread plates in triplicate. One series of plates was inoculated and incubated in an anaerobic glove box (28) containing an 80% N₂–10% CO₂–10% H₂ atmosphere; the media used in this case were anaerobic agar (BBL Microbiology Systems, Cockeysville, Md.) and medium 10 (7) modified to contain no reducing agents and only 0.04% Na₂CO₃. The plates were held in the glove box at 20 to 22°C for at least 48 h before inoculation to ensure reduction of the media. A second series of plates was prepared, inoculated, and incubated aerobically; in this case the media used were MacConkey agar no. 2 (Oxoid Ltd., Nepean, Ontario, Canada), plate count agar (PCA; Difco Laboratories, Detroit, Mich.), medium 10 modified by omission of reducing agents, resazurin, and Na₂CO₃, and Sabouraud dextrose agar (Difco). MacConkey agar no. 2 facilitates the recognition of enterococci in the presence of coliforms. Plates of both the aerobic and anaerobic series were incubated at 20 to 22°C for 5 days. Colonies derived from gut samples were then counted, and those representative of different types observed microscopically were transferred to tubes of PCA. Colonies derived from frass and bran samples were similarly isolated. Cultures were incubated at 35°C until growth was visible; some of the growth was then suspended in sterile 0.85% saline. Purity of the isolates was established by restreaking the suspensions on PCA or MacConkey agar no. 2 and reisolating typical colonies. Stock cultures of the isolates were maintained on PCA at 4°C and transferred at monthly intervals.

**Characterization of isolates.** To characterize the isolated strains, morphology and colony type on PCA, Gram stain and catalase reactions, and motility and oxygen relationship were determined by standard methods (19, 36). Gram-negative bacilli, whether fermentative or nonfermentative, were further characterized by using the API 20E system (API Laboratory Products Ltd., St. Laurent, Quebec, Canada); additional tests applied to some isolates were reaction on DNase test agar with methyl green (Difco) and capacity to grow at 42°C in nutrient broth. Gram-positive cocci were further characterized by the API 20S system; in addition, tests for hemolysis on sheep blood agar (Alpkem Western Ltd., Calgary, Alberta, Canada), esculin hydrolysis on bile esculin agar (Difco), reduction of methylene blue (MB; 0.1%) in skim milk, capacity to grow in APT broth (Difco) at pH 9.6 or 45°C, and reaction on enterococci confirmatory agar (Difco) were performed. Streptococcal group precipitin tests were done as described previously (39). All strains were tested for uric acid degradation (42), and some were tested for the capacity to produce VFA in an aqueous suspension of bran (20%) incubated for 2 weeks. Unless otherwise indicated, all tests were carried out aerobically and incubation was at 35°C; appropriate controls were included in each case.

**Determination of VFA.** Gut sections and frass which had been frozen as described above were pooled, suspended in 200 μl of distilled water, and acidified with 40 μl of 25% metaphosphoric acid. After thorough mixing, the samples were allowed to stand for 30 min and then centrifuged at 15,600 × g for 4 min. Freshly drawn pooled hemolymph was diluted with an equal volume of 0.1 M EDTA to prevent clotting, and cultures of bacteria in bran infusions were clarified; the preparations were then acidified and centrifuged as described above. VFA in the supernatants were determined by a quantitative gas chromatographic procedure (bulletin 749A; Supelco Inc., Bellefonte, Pa.), using 10% SP-1200--1% H₃PO₄ on Chromosorb W as the column packing and isobutyric acid as internal standard. Experiments showed that none of the preparations analyzed initially contained isobutyrate.

**SEM.** Whole excised guts from starved (24 h) and non-
starved grasshoppers were opened longitudinally, and the contents were removed by two gentle washes with sterile 0.85% saline. The tissue was then mounted on Styrofoam blocks and prepared for SEM as described previously for ruminal wall tissue (28). Preparations were examined in a model 505 SEM (Philips Electronics, Scarborough, Ontario, Canada), and micrographs were recorded on Polaroid 665 film.

pH. To measure the gut pH, sections removed from 12 adult insects were pooled and homogenized in 1.6 ml of CO₂-free distilled water. Measurements were made with a Radiometer PHM 64 pH meter equipped with a GK2303C combination electrode (Bach-Simpson Ltd., London, Ontario, Canada).

Egestion time. To determine the time required for digesta to pass through the gut (egestion time), two groups of adult grasshoppers were placed in individual petri plates as described above, at ambient temperature (20 to 22°C) and relative humidity (35%), and fed the normal diet of bran and barley shoots for 1 day. On day 2 residual food and frass were removed. Each insect in one group was provided with a single barley shoot (5 to 6 cm long) which had been stained with MB as a marker; the second group of insects served as controls and received unstained shoots. The time of administration of the shoots was taken as time zero, and after 1 h all insects were provided with bran. Stained shoots were prepared by immersion of the proximal end in 1% aqueous MB until the veins were uniformly colored (0.5 to 1 h), thoroughly washed with water to remove excess stain, and blotted dry. The grasshoppers were observed for a period of 8 h from time zero, and the time and duration of shoot consumption during the first hour were recorded in each case. The time of deposit of frass was also noted, and pellets deposited by insects fed stained shoots were immediately examined microscopically for presence of the marker; all insects were anesthetized and dissected, and the appearance of the contents of each gut region was recorded.

Statistical analysis. Statistical comparisons of data, involving analysis of variance and Student's t test, were calculated with Statwork software (Crickef Software Inc., Philadelphia, Pa.). Following analysis of variance, means were compared by Duncan's new multiple range test (20).

RESULTS

Three pooled samples of foregut, midgut, and hindgut, each derived from 12 adult M. sanguinipes, yielded mean pH values (± standard error [SE]) of 5.15 ± 0.07, 6.39 ± 0.03, and 5.98 ± 0.07, respectively.

Observation by light microscopy of homogenates prepared from midgut and hindgut sections showed no evidence for the presence of protozoa, and fungi were only sporadically recovered when the undiluted homogenates were plated on Sabouraud dextrose agar. A large population of bacteria was visible, although the range of morphological types present was limited. The predominant type consisted of nonmotile gram-positive cocci, which were arranged singly and in pairs, tetrads, short chains, and clusters; smaller numbers of gram-negative bacilli were also present, some of which were highly motile and showed unipolar swelling.

In cultural studies, colony counts obtained from midgut/hindgut sections on a series of aerobic and anaerobic media ranged between 5.7 and 5.9 log₁₀ colonies per mg, and those yielded by frass ranged from 5.3 to 5.5 log₁₀ colonies per mg (Table 1). Analysis of variance showed that neither the medium nor the incubation conditions used significantly affected the counts (P > 0.05). In particular, virtually identical counts were obtained on modified medium 10 under both aerobic and anaerobic conditions. These results indicate that the predominant flora of the midgut/hindgut regions was facultatively anaerobic and nutritionally nonfastidious.

A total of 173 pure strains of bacteria were isolated from the plates used to obtain these counts. Isolations were made at random from plates incubated with both low and high dilutions of the homogenates. The resulting strains were characterized phenotypically, and 168 were presumptively assigned to 11 genera and species (Table 2). These included Enterococcus spp., Serratia liquefaciens, Pseudomonas spp., and Enterobacter spp. Except for E. cloacae and Enterobacter aerogenes, of which single strains were recovered from only one grasshopper in each case, representative strains of all taxonomic groups encountered were isolated from 3 or more of the 14 grasshoppers examined. Enterococcus avium (13) and S. liquefaciens were recovered from all 14 grasshoppers. The dilutions from which characteristic colony types were isolated indicated that Enterococcus spp. associated with the gut were 2 to 3 orders of magnitude more numerous than other genera. All isolates grew aerobically, and none formed spores.

The 81 strains identified as Enterococcus spp. were all capable of growth at 45°C and pH 9.6 and in the presence of 6.5% NaCl; identification to species was based on differences in esculin and hippurate hydrolysis and in the Lancefield group D antigen reaction. All of these strains fermented trehalose, but none degraded uric acid. Gram-negative motile bacilli with fermentative metabolism were assigned to S. liquefaciens and Enterobacter spp. Isolates belonging to the former species produced DNase. Like E. cloacae, E. aerogenes, and about 50% of the Enterobacter agglomerans strains, they also degraded uric acid; the remaining E. agglomerans isolates did not do so. Gram-negative nonfermentative motile bacilli identified as Pseudomonas spp. fell into three distinct categories on the basis of the characters determined. Strains identified as Pseudomonas maltophilia, a synonym of Xanthomonas maltophilia (38), were unique in that they produced yellow cellular pigments, decarboxylated lysine, and hydrolyzed o-nitrophenyl-β-D-galactoside. These

<table>
<thead>
<tr>
<th>Table 1. Colony counts of bacteria from midgut/hindgut sections of M. sanguinipes and from frass</th>
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<table>
<thead>
<tr>
<th>Sample (no.)</th>
<th>Mean fresh wt (mg) of sample ± SE</th>
<th>Colony count (log₁₀ colonies per mg) ± SE†</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCA</td>
</tr>
<tr>
<td>Midgut/hindgut (12)</td>
<td>62.7 ± 2.5</td>
<td>5.86 ± 0.28</td>
</tr>
<tr>
<td>Frass (6)</td>
<td>31.8 ± 0.1</td>
<td>5.43 ± 0.01</td>
</tr>
</tbody>
</table>

† Plates in triplicate incubated at 20 to 22°C for 5 days.
strains, like *Pseudomonas stutzeri*, showed strain variability in their capacity to hydrolyze uric acid. The strains identified as *Pseudomonas fluorescens* were particularly active degraders of this compound. Strains representing *E. avium*, *S. liquefaciens*, and *P. fluorescens* only were recovered from frass, and strains representing *E. faecalis* (35). *Enterococcus sp.*, *E. avium*, and *E. agglomerans* (uric acid-negative strains) were recovered from bran fed to the grasshoppers; the barley shoots yielded virtually no colonies under the experimental conditions used.

A high proportion of the morphological types of bacteria seen microscopically in gut homogenates were represented among the isolates recovered from these homogenates. Clearly, many of these organisms comprised components of the luminal population. Examination of washed samples of the gut wall by SEM revealed the presence of epimural bacteria, particularly in the foregut and hindgut. In grasshoppers fed ad libitum, and in those starved for 24 h before dissection, only coccolid forms were associated with the foregut wall. Whereas massed coccolid cells were often seen in crevices of the wall (Fig. 1A), where residual food fragments were sometimes visible, there was direct and extensive attachment of cells to smooth cuticular surfaces of the wall, especially in the spinous areas (Fig. 1B). Epimural bacteria were substantially more numerous in starved than in nonstarved insects. There was little evidence of bacterial attachment to the inner surfaces of the ceca. Coccolid cells were extensively incorporated into the developing matrix of the peritrophic membrane which lined the midgut (Fig. 1C), but when this material was removed, the surface structure of the midgut appeared virtually free of attached bacteria. In a high proportion of the grasshoppers examined, spherical to slightly ovoid bodies up to 10 μm in diameter and with smooth or rough surfaces were closely associated with the microvillous surface of the midgut epithelium (Fig. 1D). Although bacterial attachment in the pyloric region was rare, an extensive population of epimural bacteria was associated with the lining of the hindgut. As in the foregut, colonization of large areas of the surface was by a single morphotype. In the ileum the predominant morphotype was coccoid (Fig. 2A), but in the rectal sac some areas were colonized by bacilli (Fig. 2B). When frass was examined by SEM, the intact peritrophic membrane surrounding each pellet was clearly visible (Fig. 3A), and bacteria, principally cocci were extensively associated with both the outer surface of the membrane and the food residues contained within it. The appearance of the residues (Fig. 3B) was little different from that of freshly ingested material.

### TABLE 2. Selected characteristics of bacteria isolated from midgut/hindgut sections of *M. sanguinipes*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of strains isolated</th>
<th>Source</th>
<th>Uric acid degradationa</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grasshoppers (n = 14)</td>
<td>Frass (n = 2)</td>
<td>Bran (n = 2)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>2</td>
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<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>9</td>
<td>13</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
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</table>

a D, Strain variable.

Analysis of the gut homogenates, hemolymph, and frass for VFA showed the presence of acetate in all components (Table 3), but no other short-chain acids were detected. There was a substantial degree of interpool variability in the concentration of acetate present in all components except hemolymph; as a result, differences between the mean VFA concentrations in foregut, midgut, and hindgut homogenates lacked significance (P > 0.05). The concentration of acetate tended to be highest in the hindgut, however, and the midgut generally contained less acetate than the foregut. When the capacity of strains representative of the bacterial groups isolated from the foregut were tested for their capacity to produce VFA in an aqueous suspension of bran, it was found that net production of acetate by *E. faecalis*, *Enterococcus sp.*, *E. avium*, and *E. agglomerans* (uric acid-negative strains) was 6.9, 11.8, 9.6, and 3.4 μmol/mg, respectively; for all other species, it was <1 μmol/mg. *S. liquefaciens* produced traces of propionate and n-butyrate in addition to acetate.

When four grasshoppers were individually offered MB-stained barley shoots, the mean (± SE) total time spent consuming the shoots during the first hour was 4.25 ± 0.59 min. In the control group offered unstained shoots, the mean total consumption time within the first hour was 8.50 ± 1.76 min, although the mean meal size within the same period differed between the groups by only 6% (P > 0.05). Microscopic observation of frass pellets showed that the marker appeared 3.0 to 5.7 h (mean ± SE, 3.9 ± 1.3 h) after feeding began. When the insects were dissected, the foregut and hindgut regions were found to contain solid digesta in which stained shoot fragments were present. The contents of the hindgut region were greenish in color, but in only one insect was solid material present; in the other three insects the hindgut contained only fluid. There was no evidence that MB was present in hemolymph.

**DISCUSSION**

The results obtained show that the gut microflora of adult *M. sanguinipes* raised on barley shoots and bran was abundant but relatively simple, predominantly consisting of a limited number of types of aerobic and facultatively anaerobic bacteria. The predominant flora was qualitatively similar to that described for other acridids (24, 37), although the range of taxonomic groups recovered was narrower than that associated with field samples of *Camnula pellucida* and other species of *Melanoplus* from western Canada (4). For example, genera such as *Brevibacterium*, *Achromobacter*,...
and Alcaligenes, which were widely distributed among these grasshoppers, were not isolated in the present study. This may reflect the narrower range of food available to laboratory-reared insects or differences in other environmental factors between field and laboratory. Enterococcus spp. and E. agglomerans, typical of strains recovered from the gut, were present on bran fed to the grasshoppers, suggesting that these components of the gut flora could have been derived directly from the diet; other species present in the gut, however, were not recovered from either bran or barley shoots. These results suggest that at least the majority of gut bacteria recovered represented the indigenous flora.

Despite the use of appropriate media and rigorous anaerobiosis, no obligately anaerobic bacteria were recovered; there were no significant differences between the counts obtained on aerobic and anaerobic media, and all strains isolated under anaerobic conditions subsequently grew readily in air. Clostridium spp. were isolated from the gut of S. gregaria (37), but using cellulose powder as the substrate and incubation in an anaerobic jar, Payne and Davidson (32) failed to detect the presence of anaerobic bacteria in the gut of this species. However, nonsporeforming anaerobic bacteria, some of which hydrolyzed carboxymethyl cellulose (15), have been isolated from the hindgut of cockroaches, Periplaneta americana and Eublaberus posticus (14, 15), and the common house cricket, Acheta domesticus (42). Electron microscopic studies have shown the presence in Periplaneta americana and Eublaberus posticus of bacteria morphologically characteristic of Methanospirillum sp. (2, 16). The redox potential in the hindgut of Periplaneta americana was reported to average -170 mV (range, -84 to -241 mV; D. Warhurst, Ph.D. thesis, University of Lei-
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FIG. 2. SEMs of surfaces of washed gut sections from M. sanguinipes. (A) Hindgut (ileum), showing microcolonies of epimural cocci on cuticular surface. Bar, 2 μm. (B) Hindgut (rectal sac), showing microcolony of epimural bacilli. Bar, 5 μm.

FIG. 3. SEMs of frass from M. sanguinipes. (A) Intact frass pellet. P, Peritrophic membrane surrounding food residues. (B) Food residues in frass. Bars, 200 μm.

Cester, Leicester, England, 1964 [quoted in reference 2]) and is therefore consistent with the presence of such bacteria.

The relative pH values in the gut of M. sanguinipes were similar to those described for other acridids (21, 29, 30), although the absolute values in the foregut and midgut were somewhat lower. Foregut pH varies with diet because, in contrast to the midgut, buffering capacity is inappreciable (9). VFA, predominantly acetate, are characteristic products in the hindgut of insects which carry an anaerobic acidogenic flora (31), and the acids are transported through the gut wall. Despite the insignificance of an anaerobic flora in the grasshopper, acetate was found in all sections of the gut, and the concentration was higher in the hindgut and foregut than in the midgut. The capacity of isolated strains of Enterococcus spp. and E. agglomerans (uric acid-negative strains) to produce acetate in a bran suspension suggests that acetate production in vivo is, at least in part, of bacterial origin. Although no attempt was made in the present study to isolate bacteria from the foregut, SEM of the foregut wall usually demonstrated the presence of an extensive population of adherent coccoid organisms. Since the cuticular lining of the foregut is relatively impermeable, even to water (26), and the contents lack buffering capacity, the accumulation of acetate in this region could account for the low pH observed. The presence of acetate in the hemolymph, however, is in accord with its absorption from other sections of gut and a putative role in insect metabolism (40). The production and absorption of acetate may therefore represent a metabolic interaction between the host insect and its gut flora.

SEM showed that the wall of the foregut and hindgut in M.
**TABLE 3. Concentration of acetate in pooled gut sections, hemolymph, and frass of M. sanguinipes**

<table>
<thead>
<tr>
<th>Pool*</th>
<th>Gut section</th>
<th>Acetate concn (μmol/mg)</th>
<th>Hemolymph</th>
<th>Frass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foregut</td>
<td>Midgut</td>
<td>Hindgut</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.92+</td>
<td>4.63</td>
<td>14.38</td>
<td>6.97</td>
</tr>
<tr>
<td>2</td>
<td>6.03</td>
<td>9.53</td>
<td>11.20</td>
<td>7.54</td>
</tr>
<tr>
<td>3</td>
<td>6.91</td>
<td>2.61</td>
<td>7.26</td>
<td>5.66</td>
</tr>
</tbody>
</table>

Mean ± SE 7.62 ± 1.17 5.59 ± 1.73 10.95 ± 2.06 6.72 ± 0.55 3.66 ± 2.18

* For each pool, n = 12.
* Frass was collected over a 5-h period of starvation prior to sacrifice of the grasshoppers.

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**LITERATURE CITED**


*sanguinipes* supported a population of epimural bacteria morphologically similar to the predominant types of bacteria recovered in culture. Although epimural bacteria were not associated with the midgut epithelium, in agreement with previous results for *Periplaneta americana* (2), bacteria were extensively incorporated into the peritrophic membrane. This acellular structure is continuously secreted from the epithelium and surrounds the digesta during their subsequent movement through the gut and their excretion (33). Ulrich et al. (42) similarly demonstrated bacterial attachment to the luminal, but not the epithelial, side of the peritrophic membrane in *Acheta domestica*. Secretion of the membrane may therefore serve as a mechanical barrier to the attachment of bacteria to the midgut epithelium by creating an ectotrophic space devoid of cells. Some organisms, however, clearly escape enclosure since the hindgut contained an extensive epimural flora. Components of the epimural population in higher animals may play specific functional roles in relation to gut physiology (34) which are dependent on their spatial distribution; there appears to be no evidence as yet of a similar role in the case of acridids.

SEM revealed the presence of spherical to ovoid structures up to 10 μm in diameter associated with the midgut epithelium. These structures were not readily identifiable from the limited visual evidence available. Though they resembled spores or cysts, they did not correspond in size or appearance with any stage in the life cycle of *Malameba locustae*, a common parasite of *Melanoplus* spp. maintained in laboratory colonies (22), or with the eugregarine parasites of *Schistocerca* and related genera (8). The possibility exists that the structures were of physiological origin, but further work is required to test such a hypothesis.

The egestion time in *M. sanguinipes* was determined by using MB-stained barley shoots. The use of stained plant material is clearly open to the criticism that feeding duration or meal size might be reduced when compared with the consumption of unstained material. Baines et al. (1), however, demonstrated that, provided the concentration of MB in shoots was not excessive, the meal size consumed by nymphs of *Locusta migratoria* was unaffected. In the present work there was no significant difference in meal size between grasshoppers fed stained and unstained shoots. Egestion time is influenced by numerous factors, including temperature, activity, prior starvation, meal size, and food type (23), and liquid from the food moves through the gut faster than the solids (1). The egestion time determined here represents the time required for the leading edge of the solid portion of the stained meal to appear in the frass, rather than the time required for the entire meal to clear the gut; for this reason it reflects the maximum rate of digesta passage under the experimental conditions used. Since the gut of acridid insects is short, one would expect egestion time to be relatively short as well. In fact, the mean egestion time obtained, 3.9 h, compares very favorably with the results of earlier work with grasshoppers (41) and locusts (1) in which different experimental conditions were used. The relatively intact appearance of the food residues in frass and the predominance in the gut of rapidly growing, facultatively anaerobic bacteria are consistent with the short egestion time.


