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Diversity and Contribution of the Intestinal Bacterial Community to the Development of *Musca domestica* (Diptera: Muscidae) Larvae

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ABSTRACT The bacterial diversity in the intestinal tract of *Musca domestica* L. was examined in larvae collected from turkey bedding and corn silage. Aerobic culturing yielded 25 bacterial species, including 11 from larvae collected from turkey bedding and 14 from larvae collected from corn silage. *Providencia rettgeri* (Hadley, Elkins & Caldwell) was the only species common to both environments. Two mammalian pathogens, *Yersinia pseudotuberculosis* (Pfeiffer) and *Ochrobactrum anthropi* (Holmes), were isolated from the larval intestinal tracts. The majority of isolates represented facultatively anaerobic heterotrophs capable of fermentation. The significance of these bacteria for development of house fly larvae was evaluated by bioassays on trypticase soy egg yolk agar. Pure cultures of individual bacterial species isolated from the intestinal tract of larvae from turkey bedding supported development of flies to a much greater extent than those isolated from larvae from corn silage. House fly development was best supported by a *Streptococcus sanguis* (White) isolate. The significance of bacteria for development of house flies is discussed.

KEY WORDS house fly, intestinal tract, bacteria, artificial medium, larval development

THE ASSOCIATION OF muscoid flies and bacteria has been studied from several perspectives, including the significance of bacteria for larval development, digestibility of bacteria in the intestinal tract of fly larvae, and the potential transmission of pathogens by adult flies. Virtually any environment rich in organic matter harbors a diverse bacterial community and becomes a suitable substrate for development of house flies and other cyclorrhaphous flies (Spiller 1964).

The significance of bacteria for development has been examined for house flies (Schmidtmann and Martin 1992, Watson et al. 1993), stable flies (Lysyk et al. 1999), and face flies (Hollis et al. 1985). Digestibility of bacteria in the intestinal tract was examined in house flies (Espinosa-Fuentes and Terra 1987) and blow flies (Greenberg 1968). Studies of morphological and physiological adaptations of muscoid flies for uptake, storage, and digestion of bacteria (Dowding 1967, Espinosa-Fuentes and Terra 1987) also emphasized the importance of bacteria in larval development. The potential of house flies to transmit pathogens such as *Helicobacter pylori* (Goodwin) (Grubel et al. 1997), *Campylobacter jejuni* (Jones) (Shane et al. 1985), *Salmonella* sp., *Shigella* sp. (Greenberg 1971), and pseudorabies virus (Zimmerman et al. 1989) has been reported.

Studies focusing on house flies reared on artificial media, including blood agar (Schmidtmann and Martin 1992) and egg yolk agar (Watson et al. 1993), have shown that larvae failed to grow in an axenic environment, indicating that bacteria contributed to the suitability of organic substrates for larval growth. The midgut of house fly larvae provides conditions and enzymes necessary for bacterial digestion (Espinosa-Fuentes and Terra 1987). Nevertheless, the composition of the bacterial community in the digestive tract of house fly larvae developing in a natural environment has not been investigated.

Our study describes the diversity of the bacterial community in the intestinal tract of house fly larvae collected from two natural environments, corn silage and turkey bedding, along with an evaluation of the importance of these bacteria in house fly development on an artificial medium.

Materials and Methods

Isolation and Identification of Bacteria. Third-instar *M. domestica* larvae were collected from decaying corn silage and used turkey bedding (wood shavings mixed with turkey feces). Larvae were transferred aseptically to sterile glass vials, transported to the laboratory, and immediately processed. Ten larvae from each substrate were ligated at the anterior and posterior ends to prevent reduction of the intestinal bacterial population during surface sterilization. Larvae were surface sterilized in 70% ethanol for 30 s, rinsed in sterile water, submerged in 0.05% sodium hypochlorite for 60 s, rinsed three times in sterile water, and divided into five samples. Each sample was two larvae homogenized in a glass homogenizer in 1 ml of sterile PP buffer (10 mM potassium phosphate, pH 7.0–7.2) and spread on a broad spectrum nutrient medium PCA (Plate Count Agar, Difco, Detroit, MI), and two differential media, HEA (Hektoen Enteric Agar, Difco) and EMB (Eosin Methylene Blue Agar,

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Table 1. Identification of bacteria isolated from the intestinal tract of house fly larvae collected from corn silage and turkey bedding

Silage isolates	Identification	Type of metabolism	Fermentation	Turkey isolates	Identification	Type of metabolism	Fermentation
MdS1	*Serratia marcescens*	f. anaerobic	+	MdT1	*Morganella morganii*	f. anaerobic	+
MdS2	*Sphingobacterium spiritivorum*	aerob/micr	−	MdT2	*Staphylococcus epidermidis*	f. anaerobic	+
MdS3	*Xanthobacter flavus*	aerob/micr	−	MdT3	NI	f. anaerobic	+
MdS4	*Microbacterium liquafaciens*	f. anaerobic	+	MdT4	*Sphingomonas capsulata*	st. aerobic	−
MdS5	*Providencia stuartii*	f. anaerobic	+	MdT5	*Bacillus* sp.	f. anaerobic	+
MdS6	*Gordona amarae*	st. aerobic	−	MdT6	*Bacillus coagulans*	f. anaerobic	+
MdS7	*Corynebacterium aquaticum*	f. anaerobic	+	MdT7	*Providencia rettgeri*	f. anaerobic	+
MdS8	*Providencia rettgeri*	f. anaerobic	+	MdT8	*Yersinia pseudotuberculosis*	f. anaerobic	+
MdS9	*Microbacterium esteraromaticum*	f. anaerobic	+	MdT9	*Lactococcus garviae*	f. anaerobic	+
MdS11	*Microbacterium lacticum*	f. anaerobic	+	MdT10	*Staphylococcus lentus*	f. anaerobic	+
MdS13	*Ochrobactrum anthropi*	aerob/micr.	+	MdT11	*Streptococcus sanguis*	f. anaerobic	+
MdS16	*Clavibacter michiganese*	st. aerobic	−				
MdS17	*Corynebacterium seminale*	f. anaerobic	+				
MdS18	*Microbacterium barkeri*	f. anaerobic	+				

f. anaerobic, facultative anaerobic; aerob/micr, aerobic and microaerophilic; st. aerobic, strictly aerobic. −, does not ferment; +, ferments; NI, not identified.

Difco) in 10^{-1} to 10^{-4} dilutions. Plates were incubated aerobically at room temperature. Morphologically different single colonies were isolated on PCA. The resulting 45 isolates were screened for differences by standard diagnostic methods (Garcia et al. 1998): cell morphology (cell size and shape) by phase-contrast microscopy, Gram staining (Fisherbrand, Pittsburgh, PA), oxidase test (BBL Oxidase, Becton Dickinson, Cockeysville, MD), catalase test (using 3% H_2O_2) and motility (examined on wet mount slide under a microscope and by stabbing of isolates into SAM (soft agar medium: tryptose 10.0 g/liter, NaCl 5.0 g/liter, agar 5.0 g/liter). Twenty-five isolates were selected and identified by FAME (fatty acid methyl esters) analysis (Microcheck, Northfield, VT) (Perry and Staley 1997). Additional API 20E tests (BioMerieux Vitek, Hazelwood, MO) were used for identification of enterics (Perry and Staley 1997).

Trypticase Soy Egg Yolk Agar Bacterial Bioassay. Trypticase soy egg yolk agar was used to ensure good growth of all isolates. Trypticase soy broth (BBL, Becton Dickinson), with agar (12.0 g/liter) (BBL, Becton Dickinson) was autoclaved, and allowed to cool to 50°C. Egg yolks (two yolks per liter) from surface sterilized large commercial chicken eggs were prepared and mixed into trypticase soy broth by the method of Watson et al. (1993). The medium was poured into petri plates and used for the bioassays. Single colonies of individual bacterial isolates were harvested by loop from 72-h-old cultures on PCA and transferred to 5.0 ml of sterile PP buffer and vortexed. Inocula of isolates were prepared in the concentration range of 1.0×10^4 to 4.3×10^4 cells per milliliter. Each isolate (100 μl) was spread in quadruplicate on trypticase soy egg yolk agar. House fly eggs, harvested from our laboratory house fly colony (larvae maintained on moistened wheat bran and calf food [13:1 ratio], adults on sugar and powdered milk [3:1 ratio] and water ad libitum) were surface sterilized (as described above for sterilization of house fly larvae) and incubated on sterile wet filter paper in a parafilm-sealed petri dish in an incubator for 24 h at 26°C. Newly hatched larvae were transferred aseptically by sterile paint brush to trypticase soy egg yolk plates with 24-h-old bacterial cultures. Five larvae per plate were used in four bioassays for each bacterial isolate. As a control group, sterile neonate larvae were transferred to axenic trypticase soy egg yolk agar plates. Plates were kept at 25°C in an incubator until pupation. All plates were monitored daily, newly pupated individuals were removed, weighed, and transferred to sterile petri plates for emergence. Trypticase soy egg yolk bioassay was replicated three times over a 6-mo period.

Mean pupal weight was calculated for each bacterial culture treatment. Means were analyzed using one-way analysis of variance (ANOVA) and compared by least significant difference (LSD) test (Minitab 1997). Correlation between means of pupal weight and emergence was assessed by Pearson correlation test (Minitab 1997).

Results

Isolation and Identification of Bacteria. Intestinal tracts of the house fly larvae collected from two different environments harbored a diverse microbial population. Eleven bacterial species of nine genera, including *Staphylococcus*, *Streptococcus*, *Lactococcus*, *Morganella*, *Providencia*, *Yersinia*, *Sphingomonas*, and *Bacillus* were isolated from the intestinal tract of *M. domestica* larvae collected from turkey bedding (Table 1). *Yersinia pseudotuberculosis*, a pathogen of humans and animals, including turkeys and pigs (Wallner-Pendleton and Cooper 1983, Fukushima et al. 1984), was isolated but not tested for pathogenicity. Isolate MdT5 was identified to the genus level (*Bacillus* sp.) and MdT3 isolate was not identifiable by FAME analysis (Table 1). Phenotypic tests of MdT3 revealed that it was a short, nonmotile, Gram-negative rod that fermented glucose and lactose.

Culturing the gut contents of larvae from corn silage yielded 14 bacterial species of nine genera, including *Serratia*, *Providencia*, *Microbacterium*, *Xanthobacter*, *Sphingobacterium*, *Corynebacterium*, *Clavibacter*, *Ochrobacter*, and *Gordona* (Table 1). *Providencia rettgeri* was the only bacterial species common to both

Table 2. Development of *M. domestica* larvae on trypticase soy egg yolk agar inoculated with different bacterial isolates from larvae collected from turkey bedding

Bacterial isolate	No. of neonates	No. of pupae	Pupal weight (mean ± SEM), mg	No. of adults	Emergence, %
Staphylococcus epidermidis	60	5	14.1[a]	0	0.0
Staphylococcus lentus	60	37	21.3 ± 0.6[b]	33	89.2
Streptococcus sanguis	60	56	20.2 ± 0.5[b]	51	91.1
Lactococcus garviae	60	47	21.6 ± 0.7[b]	43	91.5
Sphinomonas capsulata[a]	20	18	24.3 ± 2.0	8	44.4
Morganella morganii	60	0	NA	0	NA
Providencia rettgerii	60	0	NA	0	NA
Yersinia pseudotuberculosis	60	25	19.1 ± 0.7[b]	18	72.0
Bacillus coagulans	60	51	24.2 ± 0.5[c]	39	76.5
Bacillus sp.	60	8	19.5 ± 1.5[b]	0	0.0
MdT3 isolate	60	55	19.1 ± 0.5[b]	47	85.5
Control (no bacteria)	60	0	NA	0	NA

Means followed by the same letter are not significantly different, $P \leq 0.001$, ANOVA. NA, not applicable.
[a] Isolate not viable after the first bioassay.

environments. Most isolates represented facultative and aerotolerant anaerobic heterotrophs capable of fermentation (Table 1).

Trypticase Soy Egg Yolk Agar Bacterial Bioassays. The contribution of bacterial isolates to the development of house fly larvae on trypticase soy egg yolk agar medium revealed great differences among the isolates as well as the two substrates. Most bacterial isolates from turkey bedding larvae supported development of *M. domestica* to pupation (Table 2). The minimal mass of pupae from our regular laboratory colonies (18.0 mg) was reached on trypticase soy egg yolk agar inoculated with eight out of 11 isolates (range, 19.1–24.2 mg; Table 2). House fly pupae from trypticase soy egg yolk inoculated with *Bacillus coagulans* (Hammer) were significantly heavier than those from trypticase soy egg yolk inoculated with other isolates from larvae collected from turkey bedding ($F = 11.51$, df = 283, $P \leq 0.001$) (Table 2). However, emergence varied from 0 to 100% (Table 2) and correlation between pupal weight and percent emergence was low (correlation coefficient $r = 0.312$). This suggests that greater pupal weight does not necessarily indicate successful development (Table 2). Consequently, house fly development was best supported with *Streptococcus sanguis* with 85% larvae reaching adult stage. Two enteric isolates, *Morganella morganii* (Winslow) and *P. rettgeri*, did not provide conditions/nutrients for any larval growth (Table 2). In addition, only five larvae reached the pupal stage on medium with *Staphylococcus epidermidis* (Winslow) and pupal mass (14.1 mg) was significantly lower than the pupal mass of larvae reared on the other isolates (Table 2); and they did not emerge.

In contrast, development of house fly larvae was very poor on trypticase soy egg yolk agar inoculated with bacteria from larvae from corn silage. Only seven out of 14 isolates supported house fly development to some extent (Table 3). *Sphingobacterium spiritivorum* (Yabuuchi), supported larval development the best (pupation [80%], pupal weight [$F = 2.86$; df = 114; $P \leq 0.01$], emergence [92%]). The rest of the isolates contributed little to larval development (pupation range, 0–35%; emergence range, 0–70%) (Table 3). These results indicate that corn silage, despite its greater bacterial diversity, was less suitable for house

Table 3. Development of *M. domestica* larvae on trypticase soy egg yolk agar inoculated with different bacterial isolates from larvae collected from corn silage

Bacterial isolate	No. of neonates	No. of pupae	Pupal weight (mean ± SEM), mg	No. of adults	Emergence, %
Serratia marcescens	60	0	NA	0	NA
Providencia rettgeri	60	0	NA	0	NA
Providencia stuartii	60	0	NA	0	NA
Microbacterium liquefaciens	60	4	11.8 ± 1.1[a]	2	50.0
Microbacterium barkei	60	0	NA	0	NA
Microbacterium esteraromaticum	60	18	19.5 ± 1.7[b]	10	55.6
Microbacterium lacticum	60	11	18.6 ± 1.6[a,b]	8	72.7
Xanthobacter flavus	60	0	NA	0	NA
Sphingobacterium spiritivorum	60	48	20.3 ± 0.6[b]	45	93.4
Corynebacterium aquaticum	60	10	20.6 ± 2.2[b]	7	70.0
Corynebacterium seminale	60	0	NA	0	NA
Clavibacter michiganese	60	21	18.0 ± 0.9[a,b]	13	61.9
Ochrobacter anthropi	60	3	13.9 ± 0.8[a]	2	66.7
Gordona amarae	60	0	NA	0	NA
Control (no bacteria)	60	0	NA	0	NA

Means followed by the same letter are not significantly different, $P \leq 0.05$, ANOVA. NA, not applicable.

fly development than turkey bedding. Larvae in the negative control group (larvae on axenic trypticase soy egg yolk agar plates) died as first instars. No microbial contamination was observed in any bioassays.

Discussion

Gastrointestinal microbiology of insects is not an intensively investigated field, with the exception of the digestive tract of some termites (Breznak 1982, 1994; Ohkuma and Kudo 1996; Berchtold et al. 1999) and cockroaches (Cruden and Markowetz 1987; Zurek and Keddie 1996, 1998). The diversity of bacteria in the intestinal tract of house fly larvae has not been examined previously. Grubel et al. (1997) reported several bacterial species from the digestive tract of laboratory reared adult house flies, including *Serratia marcescens* (Bizio), *P. rettgeri*, *P. stuartii* (Ewing), *M. morganii*, *Pseudomonas aeruginosa* (Schroeter), and *Staphylococcus* sp. Interestingly, these bacterial species, with the exception of *P. aeruginosa*, also were isolated from the digestive tract of field-collected house fly larvae in our study. Although we have not examined the bacterial diversity of natural substrates, clear differences in the composition of the bacterial communities of larvae collected in two different environments indicated that house fly larvae were not highly selective in their ingestion of bacteria. House fly larvae usually were found several centimeters below the surface where anoxic microniches are common. This was reflected in their gut microbial community that was composed of predominantly facultatively anaerobic heterotrophs, capable of growth under both aerobic and anaerobic conditions. It is important to emphasize that the culture methods in our study focused on the diversity of aerobic and facultative anaerobic bacteria. Therefore, strictly anaerobic bacteria as well as bacteria unculturable on broad spectrum artificial media, such as PCA, were not detected.

Schmidtmann and Martin (1992) reported a great variation in pupation (0–69%) and emergence (0–54%) of house flies reared on blood agar medium inoculated with different laboratory cultures of bacteria and isolates from laboratory rearing medium. Lysyk et al. (1999) tested the significance of five bacterial species for development of stable fly larvae on an egg yolk agar and found that stable fly larvae failed to develop on medium inoculated with *Serratia marcescens* and *Aeromonas* sp. Both bacterial genera are known opportunistic pathogens of insects (Kalucy and Daniel 1972, Watson and Petersen 1991). In our study, the reason for the failure of house fly larvae to develop on trypticase soy egg yolk agar with most of the isolates from corn silage larvae is unknown, despite good bacterial growth on this medium. Clearly, pure cultures of most of these isolates were either indigestible by house fly larvae or did not provide proper nutrients for larval development or produced substances that inhibited larval growth. Microbial community (lactic acid bacteria) of properly fermented silage is unsuitable for larval development most likely because of lack of oxygen and low pH. Consequently, house fly larvae are commonly found only in spilled/spoiled silage, where the microbial community is altered by contamination and oxygen exposure. It is therefore possible that larval development in this environment depends on a relatively low number of bacterial species or metabolic interactions within this microbial community. In addition, it is conceivable that larvae in spoiled corn silage take advantage of strictly anaerobic bacterial contaminants (e.g., *Clostridium* sp.) that were undetectable by our aerobic culturing approach.

Little is known about the contribution of bacteria to the development of fly larvae. It is not known whether maggots require bacteria as a direct source of nutrients or as a source of nutritional supplements (e.g., vitamins). Bacteria possibly are needed to metabolize nutrients of natural organic substrates making them available for larval nutrition. Because the midgut region of the house fly digestive tract accommodates conditions and enzymes suitable for digestion of bacteria (Espinosa-Fuentes and Terra 1987), and microbial communities rapidly break down nutrients of various organic substrates, flies may take nutritional advantage of all the aforementioned aspects. House fly larvae likely benefit from complex metabolic interactions within a diverse bacterial community in a natural environment leading to a rapid degradation of organic material as well as a great build-up of bacterial mass.

In conclusion, the intestinal tract of house fly larvae harbors a diverse bacterial community, the composition of which depends on the type of organic substrate where fly larvae develop. Bioassays on an artificial medium confirmed that the development of house fly larvae was dependent on the presence of bacteria, although only some environmental bacteria in pure culture provide suitable nutrients/conditions for larval development. The development of house fly larvae in the natural environment is likely supported by a complex microbial community.

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