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Authors: Butler, Jerry F., Garcia-Maruniak, Alejandra, Meek, Frank, and Maruniak, James E.

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WILD FLORIDA HOUSE FLIES (*MUSCA DOMESTICA*) AS CARRIERS OF PATHOGENIC BACTERIA

JERRY F. BUTLER[1], ALEJANDRA GARCIA-MARUNIAK[1], FRANK MEEK[2] AND JAMES E. MARUNIAK[1]
[1]Department of Entomology and Nematology, P.O. Box 110620, University of Florida, Gainesville, FL 32611

[2]Orkin Pest Control, 2170 Piedmont Rd. NE, Atlanta, GA 30324

ABSTRACT

Bacteria carried by wild house flies (*Musca domestica* L.) collected near the rear entrances and dumpsters of 4 restaurants in north central Florida were identified. Live house flies were collected and individually transferred to blood agar plates for 1 h. After removing the flies, the plates were incubated overnight at 37ºC. Bacterial colonies that were morphologically distinct were isolated from other colonies by streaking onto new plates. The bacteria were identified by fatty acid analysis and sequence of their 16S rRNA gene. The bacterial isolates included 5 new bacterial records for house flies: *Acinetobacter baumanni*, *Bacillus pumilus, Cronobacter sakazakii, Methylobacterium persicinum, and Staphylococcus sciuri*. Other bacteria identified have been associated previously with house flies, including *Bacillus cereus, B. thuringiensis, Escherichia coli* 0157:H7, *Shigella dysenteriae, Staphylococcus saprophyticus,* and *Staphylococcus xylosus.* Most of the organisms recovered from the house fly are serious pathogens, known to produce diseases such as meningitis, food poisoning, diarrhea, abscesses, bloodstream infections, and hemorrhagic colitis. The possible exception is *Bacillus thuringiensis,* a known pathogen for insects that only occasionally produces allergic reactions in humans. If these organisms are not prevented from entering the food preparation and consumption areas, they could become a serious risk in the transmission of diseases.

Key Words: 16S rRNA gene sequence, fatty acid analysis. *Escherichia coli* 0157:H7, bacteria

RESUMEN

Bacterias obtenidas de moscas (*Musca domestica*) colectadas en el área de los basureros de cuatro restaurantes en la región de norte central de Florida fueron identificadas. Moscas vivas fueron transferidas individualmente a placas de Petri con agar de sangre por 1 hora. Después de retirar las moscas, las placas fueron incubadas por 16 h a 37ºC. Las colonias de bacteria con diferentes morfologías fueron aisladas de otras colonias resembrándolas por estría cruzadas en nuevas placas. Las bacterias fueron identificadas através del análisis de ácidos grasos y de la secuencia del gene del Acido Ribonucleico ribosomal 16S (16S rRNA). Las bacterias aisladas durante este estudio incluyen cinco especies que no habían sido previamente aisladas de moscas: *Acinetobacter baumanni*, *Bacilus pumilus*, *Cronobacter sakazakii, Methylobacterium persicinum* y *Staphylococcus sciuri*. Las otras bacterias ya habían sido encontradas asociadas con moscas en estudios anteriores e incluyen: *Bacillus cereus*, *B. thuringiensis*, *Escherichia coli* 0157:H7, *Shigella dysenteriae, Staphylococcus xylosus,* y S. *saprophyticus*. La mayoría de las bacterias obtenidas en este estudio son patógenas, capaces de producir enfermedades como meningitis, intoxicación alimenticia, diarrea, abscesos, infección sanguínea y colitis hemorrágica. La posible excepción es *B. thuringiensis* un patogeno de insectos que solo ocasionalmente causa reacciones alérgicas en humanos. Estos organismos, si no son prevenidos de entrar en el área de preparación y consumo de alimentos, pueden resultar ser un serio riesgo en la transmisión de enfermedades.

Translation provided by the authors.

The house fly (*Musca domestica* L.) long has been considered a pathogen carrying insect pest (Howard 1911; West 1951; Greenberg 1971). However, its classification as a "disease causing fly" is based on 5 scientific criteria (Olsen 1998), as follows: (1) its confirmed association with the foodborne pathogens *Escherichia coli*, *Salmonella,* and *Shigella*, (2) the fact that it is ecologically associated with humans (synanthropic), (3) its association with domestic environments (endophilic), (4) its equal attraction to excrements and human food sources, and (5) its communicative behavior that allows the house fly to easily move from heavily contaminated to human populated areas. House flies are capable of traveling up to 8 km in 24 h to find food and reproductive sites (Quarterman et al. 1954; Broce 1993). One of the most dangerous characteristics of the house fly is its feed-

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ing preferences because they are attracted to decaying plant and animal matter. This puts the fly in contact with pathogenic organisms present in our habitat, garbage, and animal wastes. The house fly must liquefy its food before ingesting it. This is done by placing a sponge like mouth part on the food source, and secreting saliva or regurgitated gut contents that changes some of the food to a liquid state which the fly can pump into its digestive system (West 1951; Harwood & James 1979). While feeding or resting, house flies often defecate, leaving fly specks and organisms passing through their digestive system (Sasaki et al. 2000). This is a simple mechanical transfer of microbes by a vector whose behavior places the contaminants from decayed and diseased sources directly onto the new food or host source they visit (Holt et al. 2007). The organisms associated with house flies number in the hundreds (Greenberg 1971; Olsen 1998; Nmoris et al. 2007) and commonly include dysentery-causing and tissue-infecting agents such as *Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Shigella* spp., *Escherichia coli, Bacillus anthracis*, Chlamydiales, *Corynebacterium* spp., and other parasitic organisms. The presence of house flies with these organisms in and around a restaurant often allows them access to food. The objective of this study was to identify the bacterial organisms present in or on house flies collected near the rear entrances of restaurants in Gainesville, Florida by using biochemical techniques.

Materials and Methods

House fly Collections

House flies were collected at the rear entrances and around dumpsters at 4 restaurants in Gainesville, Florida, with new nets that had been autoclaved. All handling of sample containers and nets was done with sterile, vinyl gloved hands. The collected flies were placed into sterile 150-mL containers and kept separate for each location. About 20 flies per location were collected and returned to the laboratory.

Bacterial Isolation

Flies were anesthetized with filtered CO_2 and transferred individually with sterile tweezers to 100-mm Petri dishes containing Columbia agar with 5% sheep blood (Becton Dickson Microbiology). Flies were kept alive on the plates where they moved over the surface while feeding, walking, and defecating. After either 1 or 16 h at room temperature, the flies were removed and the plates were incubated at 37°C overnight (~16 h). Bacterial colonies presenting morphological differences were picked and streaked on new blood agar plates. This colony selection and streaking process was repeated 2 more times in new plates to obtain individually isolated colonies.

Bacterial Identification

Analysis of Fatty Acids

Individual colonies presenting morphological differences were selected and placed into sterile 15-mL polypropylene tubes (Corning) containing 10 mL of sterile water. The samples were sent to the Bacterial Identification and Fatty Acid Analysis Laboratory, University of Florida, for bacterial identification by gas chromatographic (MIDI, Inc.) analysis of fatty acids (Welch 1991). Microbial identification is based on a similarity index (SI) presented as a numerical value that expresses how closely the fatty acid composition of an unknown sample compares with the mean fatty acid composition of the strain used to create the library entry listed as its match. Matches obtained were valued as follows: 0.500-0.999 with a separation of 0.100 between first and second choice = excellent match for genus and species; 0.300-0.500 with a 0.100 separation from the second choice = good match with the organism from the library, but may be an atypical strain; lower than 0.300 suggests that the sample does not match with any species from the database but indicates the most closely related species (Paisley 1997). For the current study, only samples that had matches of 0.5 or higher to the database were considered identified by this methods. Lipid composition, especially fatty acid composition, has been an important tool in determining taxonomic relationships among bacteria in the past and also recently with computers to determine the genus and species of bacteria (Shaw 1974; Slabbinck et al. 2009).

Sequence Analysis of the 16S rRNA Gene

To confirm bacteria identification, individual colonies were inoculated in 3 mL of LB media and grown in a shaker bath at 37°C for 16 h. The DNA was extracted with DNeasy columns (QIAgen). PCR amplification of the 16S RNA gene was obtained by using the following primer set: SPIR F: 5' GAGTTTGATCCTGGCTCAG 3' and SPIR R: 5' AGAAAGGAGGTGATCAGCC 3' (Rainey et al. 1992). The amplification was done with 2 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM of each primer and 1.25 units of Taq polymerase (Invitrogen) and a cycle program of 95°C/3 min, followed by 30 cycles of 95°C/30 s, 50°C/1 min and 72°C/2 min, with a final extension of 10 min at 72°C. PCR products were run in a 1% agarose gel, the DNA band was cleaned from the agarose with QIAquick Gel Extraction kit (QIAgen), and cloned in a pGEM-T Easy vector (Promega) into DH5-α competent cells (Invitrogen). Plasmid purification was done

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with QIAprep Spin Miniprep kit (QIAgen). Sequencing of the inserted DNA was done by using the vector primers T7 and SP6 with the Big Dye Terminator Sequencing kit (Applied Biosystems). The unincorporated labeled nucleotides were removed by ethanol precipitation and sent to the University of Florida Interdisciplinary Center for Biotechnology Research (ICBR) to be processed. The resulting electropherograms were analyzed, edited, and aligned in our lab with the Sequencher program (Gene Codes Corporation). The nucleotide sequences were compared to the nucleotide database by BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Bacterial Isolation

Plates that were in contact with live flies for 16 h had confluent bacterial growth that made it impossible to isolate individual colonies. However, a variety of morphologically distinct bacterial colonies were obtained on the plates that were in contact with the flies for 1 h. The morphology of the colonies varied in color, size, mucus formation, and the presence and size of a halo around the hemolitic colony in the blood agar (Fig. 1). This study did not aim to differentiate between bacteria carried externally or internally by the house fly.

Bacterial Identification

Fatty Acid Analysis of cultured isolates was conducted and only the samples with similarity indexes of 0.5 or higher to known bacteria were considered identified by this method (Table 1). The identification by sequence analysis of the 16S rRNA gene was done for all the bacterial samples isolated with the exception of *Cronobacter sakazakii*, and it was identified only by fatty acid analysis because it was not recovered for DNA extraction. A total of 11 pathogenic bacteria were identified and 8 of them were confirmed by both methodologies. Table 1 presents a list of different bacterial species identified, the % nucleotide identity with known bacteria, and the accession number of those sequences with the best nucleotide match. Five of the bacteria identified in this study had not been reported previously associated with house flies: *Acinetobacter baumanni*, *Bacillus pumilus, Cronobacter sakazakii*, *Methylobacterium persicinum,* and *Staphylococcus sciuri*. All of these bacteria are pathogenic to humans.

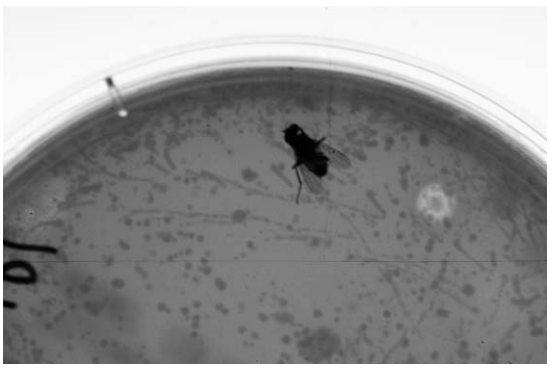


Fig. 1. Bacterial colonies obtained after placing an individual house fly on blood agar plate for 1 h followed by overnight incubation.

DISCUSSION

Bacterial samples grown from blood agar exposed to wild house flies were identified as a number of serious disease causal organisms both by fatty acid analysis and 16S rRNA gene sequences. Microbial identification by the analysis of cellular fatty acid methyl esters (MIDI-FAME) was first applied to bacteria (Shaw 1974), and also for other organisms such as yeast and fungi (Sasser 1990; Graham et al. 1995). More recently, it has been used to survey bacteria in recycling process conditions (Namjoshi et al. 2010). Previous publications indicate a threshold of 97% nucleotide identity separates bacterial species based upon the 16S ribosomal gene (Stackebrant & Goebel 1994). Although this was not an exhaustive isolation experiment, 5 bacterial species not previously associated with house flies were identified by the tested methods. This study confirmed the health risks linked with house flies and their feeding preferences since they were collected near the rear entrances and dumpsters of restaurants. The risk to the public is determined by the presence of contaminated flies visiting food within the restaurant. The number of bacteria carried by house flies from contaminated to clean surfaces has been found to range from 50 to 50,000 (De Jesus et al. 2004). The bacteria not only can be transported externally but also internally, most often in the gut and crop of the house fly (Sasaki et al. 2000; Holt et al. 2007). The amount of time and the conditions under which contaminated food are maintained is important in allowing the bacteria to grow to large numbers. The susceptibility of individuals exposed to an organism varies with their challenged immune condition or prior exposure. The presence of house flies in restaurants is real and disturbing.

Of the bacterial organisms carried in and on wild house flies found near the back entrances and dumpsters of restaurants, all but 5 have been reported as carried by house flies by others (Greenberg 1971; Levine & Levine 1991; Iwasa et al. 1999; Sasaki et al. 2000; Sulaiman et al. 2000;

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TABLE 1. IDENTIFICATION OF BACTERIA ISOLATED FROM WILD HOUSE FLIES (*MUSCA DOMESTICA*).

Bacteria Identification	Fatty acid SI[1]	16S rRNA sequence identity (%)	GenBank accession number[2]
Acinetobacter baumanni[3]	0.618	99	CP001172.1
Bacillus cereus	0.884	100	CP001177.1
Bacillus pumilus[3]	0.888	99	AE221329.1
Bacillus thuringiensis	0.845	99	AM778997.1
Cronobacter sakazakii[3]	0.879	N/R[5]	
Escherichia coli 0157:H7	0.814	99	CP001368.1
Methylobacterium persicinum[3]	N/I[4]	98	AB252202.1
Shigella dysenteriae	0.856	98	CP000034.1
Staphylococcus saprophyticus	0.799	99	AP008934.1
Staphylococcus sciuri[3]	N/I[4]	99	NR025520.1
Staphylococcus xylosus	0.772	99	G0222240.1

[1]SI = similarity index. Only samples with values over 0.500 are presented.
[2]best match by BLASTn.
[3]not previously identified in house flies.
[4]N/I: not identifiable by fatty acid analysis.
[5]N/R: not recovered for DNA analysis.

Kabkaew et al. 2000; Olsen & Hammack 2000; Nmorsi et al. 2007). The most prominent is the general presence of *Escherichia coli* specific serotype 0157:H7 with its known human risk (Kobayashi et al. 1999; Ahmad et al. 2007). The bacteria identified from house flies in this project, listed below, have the potential to produce the following problems (Greenberg 1971; Lennette et al. 1985; Howard et. al. 1987; Forbes et al. 2002; Quinn et al. 2002; Kato et al. 2008).

Acinetobacter baumanni can cause bacteremia, pneumonia, upper respiratory diseases and pulmonary disease in infants, urethritis, disease of newborn, complications of instrumentation and surgery, complications of burns, complications of compromised patients. This is a new record for house flies.

Bacillus cereus is associated with food poisoning, emetic and diarrheal type. The diarrheal type is commonly associated with meat sauces. Emetic type is almost exclusively associated with rice dishes. Wound infection, clinical infections, and human infections occur. This is a new record for house flies.

Bacillus pumilus causes food poisoning. The diarrhea type is commonly associated with meat sauces. Wound infection and human infections occur. This is a new record for house flies.

Bacillus thuringiensis produces a toxin used as an insecticide. These are common bacteria used in biological control of insects. This organism has, however, been implicated in some human infections according to Lennette et al. (1985).

Cronobacter sakazakii is common in sputum, pneumonia, lung abscess, and intestinal infections in humans and animals. It is most common in urinary tract, pulmonary, and bloodstream infections, and can be a rare cause of neonatal meningitis and sepsis. In general, this group is noted in peritonitis, bacteremia, diarrhea, enteric fevers, typhoid fever, meningitis, endocarditis, intoxication, pyelonephritis, cystitis, nosocomial infections in pediatrics, newborn, and infections in homosexual men.

Escherichia coli is associated with 4 types of human enteric disease, including enteropathogenic (diarrhea, mostly in infants), enterotoxigenic (secretory diarrhea by elaboration of heat-labile, heat stable enterotoxins or both, causing profuse watery diarrhea, and are often implicated in cases of traveler's diarrhea), enteroinvasive and hemorrhagic (dysentery-like illness similar to Shigella infections). Hemorrhagic colitis is a recently recognized enteric infection due to *E. coli* strains of a specific stereotype 0157:H7. These strains cause severe diarrhea characterized by grossly bloody stools. Organisms can be obtained from partially cooked hamburgers and are commonly isolated from house flies associated with livestock manure.

Methylobacterium persicinum is not pathogenic and it may be a contaminant in food-processing environments.

Shigella dysenteriae is primarily a human pathogen causing severe cramping, abdominal pain, and diarrhea with blood and mucus.

Staphylococcus sciuri has been associated mostly with wound infections although it can be isolated from other infections such as urinary tract infections and endocarditis.

Staphylococcus saprophyticus is a common human pathogen that causes bacteremia and infective endocarditis, infection of shunts and intravenous catheters. It is one of the most common causes of urinary tract infections in young sexually active females and urethritis in males.

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Staphylococcus xylosus is a rare pathogen or undetermined pathogen often obtained through contact with animals.

The majority of the bacteria recovered from the house fly were human pathogens with the possible exception of *Bacillus thuringiensis* that is a known insect pathogen and *Methylobacterium persicium* that is a contaminant in food preparation areas. The bacteria described in this study were obtained from house flies near areas where food processing occurs, presenting a potential human risk if house flies are not prevented from entering the food preparation and consumption areas. The bacterial organisms were recovered through contact of collected flies with culture media. Contact could have been from the surface of flies, from fly feeding and regurgitation, or defecation by flies.

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