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Attractancy potential of culturable bacteria from the gut of peach fruit fly, *Bactrocera zonata* (Saunders)

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Abstract Bacteria were isolated from the midgut of laboratory and field adult flies of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), a widely distributed fruit crop pest in India. Nine bacteria were isolated and identified as members of genera *Bacillus*, *Enterobacter*, *Klebsiella* and *Stenotrophomonas* based on culture-dependent and 16S rRNA gene sequence analysis. The results showed that bacterial diversity in the gut of *B. zonata* adults differed between the laboratory and field flies but *Stentrophomonas maltophilia, Enterobacter cloacae* and *Klebsiella pneumoniae* were present in both populations. A significant difference was found in the attractancy between the different age groups when flies were fed with protein or were protein-starved. Among bacteria, *E. cloacae* and *K. pneumoniae* had the highest attraction to flies over other bacteria across all age groups of flies. This study provides the first description of the gut microbial community of *B. zonata* and their role as attractants.

Keywords 16S rRNA · *Enterobacter cloacae* · Peach fruit fly · Bacterial diversity · Attractancy

Introduction

Insects are highly abundant and diverse species, surviving successfully in various niches on earth (Park *et al.* 2007). Most insects are associated with many different kinds of microscopic life forms, including bacteria, viruses, fungi, protozoa and other multicellular parasites (Douglas 1998). During the long period of coevolution, both groups have developed many different interactions ranging from mutualism to parasitism – for the host or the microorganism or both. Among insects, termite gut bacteria have been studied in great detail (Husseneder 2010) and recently bacteria from intestinal tracts of several insects have also been reported (Douglas 1998; Rinke *et al.* 2011; Wang *et al.* 2013). The digestive tract of the insects contains a complex biota, comprising both resident and transient members of microorganisms. Insects rely on microorganisms for various physiological functions, including digestion, nutrition, nitrogen fixation, and pheromone production (Dillon *et al.* 2002; Dillon & Dillon 2004). One of the most striking interactions is that bacteria have extended the nutritional range of insects by supplying nutrients as endosymbionts. The nutritional contributions of the gut microbiota may increase host insect survival under sub-optimal dietary conditions. They may improve digestion efficiency and provide digestive enzymes or vitamins (Nakabachi & Ishikawa 1999) which are ultimately

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manifested in the development, fecundity, and survivability of their insect hosts.

Symbiotic relationships between tephritids and their gut symbiotic bacteria have been studied particularly in a few genera, *viz., Anastrepha*, *Bactrocera*, *Ceratitis* and *Rhagoletis* (Diptera: Tephritidae). The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is distributed throughout the tropics and subtropics of the world. Despite its wide occurrence and economic importance as a fruit pest, surprisingly little is known about the bacterial communities in their guts which might play an important role not only in survival of the fruit fly but also in attracting the other fruit flies to the infected fruits, as hypothesized by many workers (Epsky *et al*. 1998; Lauzon *et al*. 1998; MacCollom *et al*. 2009; Narit & Anuchit 2011). Study of the diversity and taxonomy of these complex microbial communities native to the gut has been made possible by recent advances in molecular biology techniques, including sequencing of molecular markers like 16S rDNA.

To the best of our knowledge, there are no previous studies on the gut bacterial communities of *B. zonata*. An understanding of the microbial community structure of the fruit fly midgut will enable us to identify the organisms that play significant roles in the interaction of the insects with the hosts. The present study was undertaken to isolate, characterize, and identify the dominant bacteria inhabiting the gut of laboratory-reared and field-collected adult flies of *B. zonata* using culture-dependent methods and molecular techniques (16S rRNA) for identification of the isolates. This study also evaluated the attraction of different age group adults to the bacterial filtrates in the laboratory. This information will provide the basis for subsequent studies on the roles of these microorganisms in fruit fly development, ecology, and management.

Materials and methods

Insect source and rearing The culture of *B. zonata* was started with infested fruits collected from the Horticulture Garden, IARI (Indian Agricultural Research Institute), New Delhi, and subsequent rearing was carried out at 25±1°C and 70±5% r.h. with a natural photoperiod in the climate-controlled room. The infested fruits were kept individually in 20 × 15 cm glass jars with 10 cm of thick sterilized sand until emergence of the adults. Then, emerged adults were identified and a pair (male and female) was released into a rearing cage (30×30×30 cm) provided with fruit pulp in petri dishes wrapped in parafilm for oviposition. The feeding was supplemented with adult diet and water *ad-libitum* through soaked cotton swabs in a 100 ml conical flask. More than 15 inbreeding generations of *B. zonata* were reared in the laboratory on maggot diet for ensuring predominant and closely associated bacterial symbionts isolation. The field population were collected (as adults) from the mango orchard of IARI. Each time, the insects were brought to the laboratory and dissected.

Dissection of intestines and culturing of gut microbial communities The bacteria were isolated from the gut of laboratory reared (LR) and field collected (FC) adults of *B. zonata* as described by Lloyd *et al*. (1986). At least five adults of each sex of both colonies were used for isolation. Flies of individual colonies for dissection in glass vials were kept in a refrigerator at 4°C for 5 min to cold-anesthetize, which prevented them from regurgitating gut fluid. The adult flies were then surface sterilized by immersion in 70% ethanol for 30 sec followed by a dip in 0.25% sodium hypochlorite for 1 min and then washed thrice with sterilized distilled water (SDW) to remove the external contamination of microorganisms. The surface-sterilized flies were individually dissected in sterile agar-agar plates containing physiological saline under laminar air flow using a stereomicroscope. The midgut portion of the dissected alimentary tract was carefully separated without much damage and later squeezed with a sterile glass rod into a single droplet of SDW on a sterile glass slide to prevent its dilution. A loopful of squeezed gut fluid was streaked separately on tryptic soy agar (TSA) and nutrient agar (NA) media in petri dishes and incubated at two sets of temperature conditions (28±2°C and 37±2°C) for 24–48 h. At the end of the incubation period, each of the bacterial isolates was separated with the inoculation loop and spread onto respective TSA and NA plates for their growth. Predominant bacterial isolates were obtained through repeated sub-culturing to ensure their purity. These purified individual bacterial isolates are preserved in agar slants for further utilization. Tenfold dilutions were prepared from the entire digestive tract of both LR and FC adult flies of *B. zonata* and plated on NA medium and incubated at 30 ± 1°C for 24 h.

Morphological and biochemical characterization All bacterial isolates were first screened based on (*a*) colony

characteristics (color, size, shape, opacity, margin, elevation and viscosity); and (*b*) morphology and arrangement of cells; these isolates were identified using Bergey's Manual of Determinative Bacteriology (Whitman *et al*. 2012). The biochemical characterization of each bacterial isolate was carried out with HiAssorted™ biochemical test kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and other standard biochemical tests (Cappuccino 2005). A single and well isolated colony was picked up and inoculated into 5 ml nutrient broth and incubated at 37°C for 24–36 h. Each well in the kit was inoculated with 50 μl of the bacterial culture inocula by surface inoculation and kept for incubation at 37±1°C for 18–24 h. At the end of the incubation period, a series of reagents were added as per the manufacturer's instructions to conduct the biochemical tests.

DNA extraction and PCR amplification of 16S rRNA genes Genomic DNA was extracted from 2 ml Luria Broth (LB) overnight cultures of individual isolates grown at 30°C using the ZR fungal/bacterial DNA kit (Zymo Research Corporation, Orange, CA, USA, cat. no. D6005) following the manufacturer's instructions. Genomic DNA obtained was visualized after electrophoresis in a 1.0% agarose gel in 1× TBE buffer to assess their integrity and then stored at –80°C prior to PCR amplification.

PCR amplification of 16S rRNA gene sequencing of the isolates was done using the universal bacterial primers 11F (5'-GTTTGATCCTGGCTCAG-3') and 1512R (5'-ACGGYTACCTTGTTACGACTT-3'). The final reaction volume of 50 μl contained 1 μl each (2 mM) forward and reverse primers, 10 μl of GoTaq Buffer, 2.5 μl of $MgCl_2$ (25 mM), 1 μl of dNTP, 2 μl (500 ng) template DNA and 0.25U of Taq polymerase. The PCR was carried out using a Bio-Rad My cycler as follows: Initial denaturation at 94°C for 5 min followed by 32 cycles of 94°C for 1 min, 56°C for 1 min and extension at 72°C and final extension at 72°C for 10 min. DNA sequencing of purified 16S rRNA PCR product was carried out by Xcelris Labs Ltd., Ahmedabad, India, using Sanger's di-deoxy nucleotide sequencing method; sequences obtained were compared with those in the GenBank database using the BLAST search program (http://www.ncbi.nlm.nih.gov/). Phylogenetic analysis was performed with program MEGA 5.0 (Molecular Evolutionary Genetics Analysis, Version 5.0) (Tamura *et al*. 2011) and phylogenetic trees were inferred using the neighbor-joining method (Saitou & Nei 1987).

Transmission Electron Micrograph analysis The surface-sterilized adult flies were individually dissected with sterile scalpels and small forceps in a sterile physiological saline solution to remove the midgut portion of the alimentary tract without much damage and later squeezed the midgut content and transferred it into the eppendorf tubes aseptically. A sample was fixed in suspension with 2% glutaraldehyde (in 0.1 M buffer) for 1 h and centrifuged at 3000 rpm for 5 min to form a pellet. Cells were resuspended in 0.1M sodium phosphate buffer and washed three times and then centrifuged again to form a pellet. The sample was postfixed with 1% OsO_4 for 1 h at room temperature followed by washing the cells three times with buffer. All the samples were embedded (Embedding medium No.3: Epon 812, Araldite 6005, DDSA, dibutyl phthalate, DPM 30) in 2% agar or agarose and later cut into 1 mm^3 pieces. Agar blocks were dehydrated in 30% acetone for 15 min. Infiltration procedure was carried out with a 1:1 ratio of pure resin and acetone and the blocks were left to infiltrate overnight at 4°C or until they sank to the bottom of the vial. Again the samples were embedded in fresh resin in BEEM capsules. The polymerization step was done by placing the samples at 45°C for 12–18 h. Thin sections (80 nm) were stained with uranyl acetate and lead citrate and examined under a Zeiss EM900 transmission electron microscope (TEM) at 80 kV.

Attraction of flies to bacterial culture

Bacterial culture supernatant preparation The bacterial isolates from the gut of *B. zonata* were transferred to 10 ml of sterile nutrient broth (NB; peptone 5 g, beef extract 3 g, distilled water 1 *l*, pH adjusted to 7.0±0.2). The test tubes were incubated in a shaker at 150 rpm for 144 h at 30±1°C. Bacterial cultures were centrifuged at 10,000 rpm for 15 min and separated into pellet and supernatant. The pellet was discarded because the experiments indicated that the pellet, which contained most of the bacterial cells, was less attractive, as described by Robacker *et al.* (1993).

Attraction bioassay using rotating olfactometer cage To test the attractiveness of the bacterial culture filtrate (supernatant), different age groups (0–2, 6–8 and 20–

30 days old) of *B. zonata* flies were bioassayed. The olfactory cage used in the attraction bioassay was a modification of the laboratory testing procedures of Jang & Nishijima (1990), Narit & Anuchit (2011) and Sood *et al.* (2010). There were nine treatments consisting of six bacterial filtrates and three controls, *viz*., media blank (also incubated to insure sterility of the medium), yeast autolysate (protein source) and sterilized distilled water.

An indoor manually rotating olfactometer cage was used to evaluate the attractiveness of bacteria cultures to peach fruit flies. It is a wooden box with glass on the front side and a holder for fixing a rotating hub at its ceiling. The rotating hub consisted of a broad central tube to which glass bulbs of 100 ml capacity can be hung. This whole setup was fitted over the top of a cage. The arms of the hub unit were revolved manually every hour to ensure the orientation of flies towards the attractant source. To test for attraction of *B. zonata* adults, 0.5 ml filtrate of each bacterium was poured into the glass bulbs. After that, these glass bulbs were closed with a lid for at least 1 h before conducting the experiment. This procedure was carried out for complete emission of volatiles from the test cultures. The bulbs with treatments were attached randomly to hub arms. Approximately 50 flies (1:1) for each treatment combination were released into the cage one hour before the start of experiments. At the end of each test, flies were ejected and counted for net attractancy. Each experiment was replicated three times.

Data analysis The statistical analyses of the results were performed using the SAS statistical software. The number of fruit flies oriented towards the treatment sources in the adult bioassay cages was converted into percentages and compared by one-way analysis of variance (ANOVA), after the results had been square-root-transformed to meet the homogeneity of variances. Significant ANOVAs were followed by t-tests for multiple comparisons of mean ($P \leq 0.05$).

Results

Attempts at bacterial isolation from the midgut of *B. zonata* resulted in identification of different kinds of bacteria belonging to various genera. Rods of both Gram positive and Gram negative were the major group of bacteria found in the midgut of both colonies of *B. zonata*, represented by seven isolates (out of nine) obtained by culture-dependent techniques. The test results of the biochemical reactions mentioned above were compared with the standard ready reckoner chart provided along with the biochemical kit for generic identification of the Gram negative bacteria and with Bergey's Manual (supplementary Table 1). All the bacteria were tentatively identified as *Klebsiella*, *Enterobacter, Stenotrophomonas* and *Bacillus.* There was a considerable difference in the total number of intestinal bacteria of LR and FC adult flies of *B. zonata*. The total bacterial count was higher (2.1×10^5 cfu) in FC flies than in LR flies (1.93×10^5 cfu).

Based on 16S rRNA gene sequencing analysis, the bacterial genera identified include *Klebsiella*, *Enterobacter* and *Bacillus* as the most representative in both colonies of *B. zonata* (Table 1). Three bacterial isolates, *viz*., *Stenotrophomonas maltophilia, Klebsiella pneumoniae* and *Enterobacter cloacae* were common representatives of both populations of *B. zonata*. Bacterial isolates *B. cereus* and *B. subtilis* were isolated only from the FC flies, whereas *Enterobacter asburiae* was specific to LR flies. Figure 1 shows the phylogenetic relationship of the Gram-positive and Gram-negative bacteria found in the gut of LR and FC adult flies of *B. zonata*. Sequences obtained were submitted to the NCBI GenBank for accession numbers. These numbers include JX170711 to 170714 for LR-isolated bacteria and JX181659 to 181663 for FC-isolated bacteria. A TEM micrograph of the midgut contents of *B. zonata* indicated the presence of bacteria with varied form and shape within the gut (supplementary Fig. 1). Rod-shaped bacteria were found to be dominant inside the midgut.

Statistical analysis revealed considerable variation in mean attractancy of adult *B. zonata* flies to sources of bacterial attractants. Significant difference was found in the attractancy among the different age groups (0–2, 6–8 and 20–30-day-old adult flies) when flies were fed with protein or were protein-starved (Table 2). The 0–2-day-old newly emerged and protein-fed or protein-starved flies showed greater mean orientation towards the bacterial source with higher attraction than control treatments (media blank, yeast autolysate and autoclaved distilled water). Under protein-fed conditions, *E. cloacae*, *K. pneumoniae* and *S. maltophilia* were the most attractive species within several treatment combinations. The mean attraction of flies for these species was 3.20 (±0.23 SE), 3.11 (±0.57 SE) and 3.05 (±0.14

Table 1 Identification of the bacterial groups isolated from the gut of the peach fruit fly, *B. zonata* based on the 16S rRNA gene sequencing

Strain	Accession number	Species Identified	Length of 16 S rRNA gene sequenced (bp)	Similarity (%) with the nearest neighbour in NCBI database
Lab reared flies				
BZL1	JX170711	*Enterobacter asburiae*	1412	99
BZL2	JX170712	*Stentrophomonas maltophilia*	1407	99
BZL3	JX170713	*Klebsiella pneumoniae*	1156	99
BZL4	JX170714	*Enterobacter cloacae*	1118	100
Wild Flies				
BZW1	JX181659	*Bacillus cereus*	1158	100
BZW2	JX181660	*Enterobacter cloacae*	1174	100
BZW3	JX181661	*Bacillus subtilis*	1242	97
BZW4	JX181662	*Klebsiella pneumoniae*	1214	99
BZW5	JX181663	*Stentrophomonas maltophilia*	1419	99

SE) flies, respectively (F=5.23; *df* = 8, 26; *P*<0.05). Although the bacterium *E. cloacae* had the highest attractancy to protein-starved flies 2.76 (±0.16 SE) compared with other bacterial filtrates, the attractancy was non-significant between the treatments under olfactory cages (F=1.52; *df* = 8, 26; *P*<0.05).

Reproductively immature and protein-starved flies 6–8 days old (F=8.51; *df*= 8, 26; *P*<0.05) were more attracted to *E. cloacae* and *K. pneumonia,* with mean orientation of 4.73 (±0.37 SE) and 4.67 (±0.59 SE) flies, respectively. The response of the protein-fed flies was found to be similar (F=11.85; *df* = 8, 26; *P*<0.05), but the mean attraction was slightly decreased because of its feeding history on a complete diet (Table 2).

The mean net attractancy was highest for *E. cloacae* (3.71±0.37 flies) followed by *K. pneumoniae* (3.45 ±0.22 flies) and the least was for *B. subtilis* (2.50±0.17 flies). The intermediary response of reproductively mature flies to bacterial filtrates was *S. maltophilia* (2.76 ±0.42 flies), *E. asburiae* (2.66±0.54 flies); and 2.58 ±0.13 flies for *B. cereus* in the absence of protein in diet (F=10.00; *df* = 8, 26; *P*<0.05). A similar type of behavior was observed for the protein-fed flies (F=5.20; *df* = 8, 26; *P*<0.05). Overall, the mean levels of attractancy were significantly higher to bacterial sources than to control treatments (media blank, yeast autolysate and water) (Table 2). Among bacteria, *E. cloacae* and *K. pneumoniae* had the highest attractancy to flies,

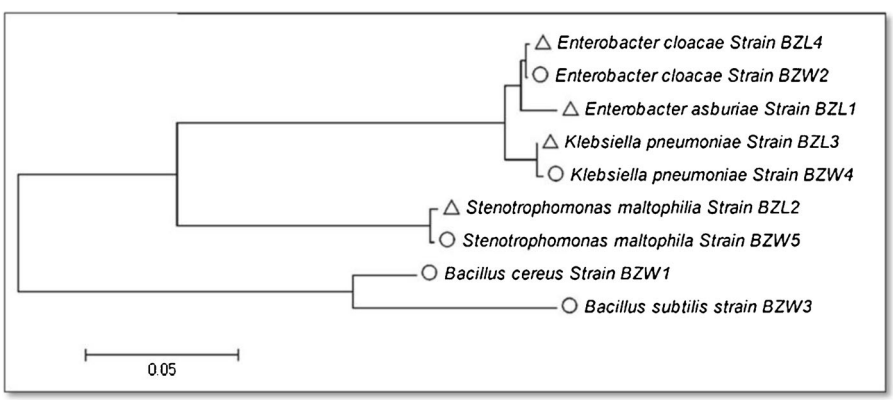

Fig. 1 Phylogenetic tree constructed using 16S rRNA gene sequence showing relationship between isolates of LR and FC adult flies of *B. zonata*. Δ = Isolated from LR flies; O = Isolated from FC flies

Table 2 Attraction of peach fruit fly, *B. zonata* of different age groups to various bacterial culture filterates in olfactory cage

Bacterial isolates	Protein fed flies			Protein starved flies		
	0-2 days	6-8 days	20-30 days	0-2 days	6-8 days	20-30 days
Enterobacter cloacae	3.20±0.23^{a}	3.32±0.39^{ab}	3.29±0.31^{a}	2.76±0.16^{a}	4.73±0.37^{a}	3.71±0.37^{a}
Klebsiella pneumoniae	3.11±0.57^{a}	3.92±0.18^{a}	2.81±0.47^{ab}	2.11±0.67^{ab}	4.67±0.59^{a}	3.45±0.22^{a}
Stenotrophomonas maltophilia	3.05±0.14^{a}	3.15±0.18^{b}	2.82±0.17^{ab}	1.73±1.00^{ab}	3.29±0.31^{b}	2.76±0.42^{b}
E. asburiae	2.68±0.27^{a}	2.35±0.48^{c}	3.40±0.46^{a}	1.99±0.43^{ab}	3.11±0.57^{b}	2.66±0.54^{b}
Bacillus subtilis	2.52±0.40^{a}	2.74±0.27^{bc}	2.28±0.28^{b}	2.05±0.62^{ab}	3.10±0.19^{b}	2.50±0.17^{b}
B. cereus	2.68±0.48^{a}	3.01±0.38^{b}	2.82±0.59^{ab}	1.77±0.96^{ab}	3.40±0.26^{b}	2.58±0.13^{b}
Media Blank	0.80±0.42^{b}	1.22±0.65^{d}	1.68±0.24^{c}	1.14±0.59^{b}	1.52±0.11^{c}	1.63±0.11^{c}
Yeast autolysate	1.32±0.68^{b}	1.38±0.21^{d}	1.24±0.63^{c}	1.15±0.58^{b}	1.55±0.36^{c}	1.66±0.36^{c}
Water	0.80±0.42^{b}	0.47±0.47^{e}	0.47±0.47^{d}	0.00^{c}	0.94±0.47^{c}	0.33±0.33^{d}

Means followed by the same letters do not differ significantly at P=0.05

The values are square root transformed value

followed by *S. maltophilia*, *E. asburiae*, *B. subtilis* and *B. cereus* across all age groups of flies.

Discussion

To the best of our knowledge, this study is the first of its kind to explore bacterial diversity within the gut of *B. zonata,* a very important pest prevalent in Southeast Asia and responsible for severe damage to its host fruit crop (Kapoor 2005). Based on BLASTn analysis, nine bacterial isolates belonging to four genera were identified, of which seven were Gram-negative bacteria to gamma proteobacteria and the two others were Gram-positive to phylum firmicutes. The Gram-negative bacterial isolates were members of genera *Enterobacter* and *Klebsiell*a (Enterobacteriaceae) and *Stenotrophomonas* belongs to Pseudomonadaceae, while the Gram-positive genus *Bacillus* belongs to Bacillaceae. Our culture-dependent studies showed that the LR and FC adult flies of *B. zonata* were significantly different in the total number of the bacterial population. This difference is due to the fact that under laboratory conditions, the flies were reared in sanitary and controlled conditions with feeding on a mixture of sterilized sucrose and yeast powder, whereas field-collected flies fed on a natural diet and were exposed to the external environment. Thus, FC flies had more diverse gut flora than LR flies.

Diverse bacterial species across the gut of tephritid flies have been studied by various workers. The genera *Enterobacter*, *Klebsiella*, *Stenotrophomonas* and *Bacillus* identified in this work were found to be associated in other tephritid species (Kuzina *et al*. 2001; Thaochan *et al*. 2010; Wang *et al*. 2011). These findings show that such bacteria are widespread in tephritids, suggesting a stable association with these fruit flies (Jang & Nishijima 1990; Lauzon *et al*. 2009; Wang *et al*. 2011). The isolation of *E. asburiae* was reported in the Oriental fruit fly, *B. dorsalis,* recently by Wang *et al*. (2013). The common bacterial species *E. cloacae*, *K. pneumoniae* and *S. maltophilia* of two populations of *B. zonata* suggest their stability across the generations and environment. Among the bacteria isolated, *Bacillus* is the most commonly found genus in the guts of many insects and is reported to be a common inhabitant of soil, water and decomposing organic matter. These bacteria might have been ingested by the fruit flies with their diet and the bacterium adapted itself to the gastrointestinal environment, forming a symbiotic association with the fruit flies (Sood & Nath 2005).

In this study the flies responded actively towards the bacterial filtrates by walking and kept flying within the bulb containing it; a significant difference was observed in this behavior as compared with control treatments. Drew & Lloyd (1989) hypothesized that bacterial odor elicits innate foraging behavior in fruit flies in search of proteinaceous food. The present findings are in line with those of Wang et al. (2013), who also reported greater attraction of adult flies of *B. dorsalis* to the bacteria *B. cereus*, *E. cloacae* and *E. faecalis* in laboratory bioassay experiments. Shi *et al*. (2012) also observed

attraction of *B. dorsalis* adult flies to isolated gut bacteria *K. pneumoniae*, *K. oxytoca* and *Raoultella terrigena* in both laboratory and field studies. Jang & Nishijima (1990) proved experimentally that *E. cloacae* was significantly more attractive than *K. pneumoniae*, *K. oxytoca*, *E. agglomerans*, *C. freundii* and *Hafnia alvei* when tested against *B. dorsalis* adult flies in an olfactometer cage.

Our results were similar to previous studies that showed an increased attraction of 10–15-day-old adult flies of *B. cucurbitae* and *B. papayae* to the odor of the bacterium *E. cloacae* as the amount of protein in the diet decreased (Narit & Anuchit 2011). An increased attraction of female flies of *B. zonata* – than male flies – was observed to bacterial attractants across all age groups. The findings of Narit & Anuchit (2011) showed that the presence or absence of host fruit resulted in similar responses to bacterial odor from protein-fed and mated flies as to virgin ones, especially in the case of *B. papayae*. Newly emerged flies of *B. zonata,* either protein-fed or protein-starved, resulted in similar orientation to bacterial filtrates perhaps because of their nutritional fulfillment during the maggot stage.

Robacker (1991) and Robacker *et al.* (1991) concluded that protein hunger brought on by yeast hydrolysate deprivation was responsible for the attractiveness of bacterial odor *Staphylococcus aureus* to *A. ludens*. Other than protein consumption, Robacker & Garcia (1993) reported that the sugar deprivation in *A. ludens* also had a higher influence on attraction to *S. aureus* compared with protein-deprived flies. The carbohydrate hunger is critical for immediate survival and the need for energy overrides the need for protein; they further concluded that after a sugar meal the flies resumed their protein-foraging behavior. Based on these experimental results one can even conclude that the bacteria culturing media (nutrient broth) could also influence the attraction of flies, as it is able to provide carbohydrates.

The bacterial species are known to produce volatiles as a metabolic by-product when cultured on medium (Kai *et al* 2009). Robacker & Bartelt (1997) sampled *K. pneumoniae* and *C. freundii* cultures by solid phase microextraction and identified chemicals attractive to *A. ludens*. Robacker & Flath (1995) identified and demonstrated the attractiveness of ammonia, several amines, imines, pyrazines and acetic acid to *A. ludens* using the headspace technique. These bacteria-emitting volatiles should be strong enough to compete with other host plant parts for their role in effective attraction of flies in the field. This suggests that combining different lures, such as host fruit volatiles with attractive bacterial cultures or ammonium acetate, may increase fly capture rate.

Our study provided the information on the culturable bacterial diversity associated with *B. zonata* which will prove useful for the eco-friendly management of this devastating insect, as these bacterial symbionts play an important role in the life cycle of fruit flies. The information on bacterial odor preference behavior of the flies may assist in finding improved fruit fly control strategies when compared with the utilization of traditional chemical control measures.

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References

Cappuccino, J. G. (2005). *Microbiology: A laboratory manual*. India: Pearson Education.

Dillon, R. J., & Dillon, V. M. (2004). The gut bacteria of insects: Nonpathogenic interactions. *Annual Review of Entomology, 49*, 71–92.

Dillon, R. J., Vennard, C., & Charnley, A. (2002). A note: Gut bacteria produce components of a locust cohesion pheromone. *Journal of Applied Microbiology, 92*, 759–763.

Douglas, A. (1998). Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology, 43*, 17–37.

Drew, R. A. I., & Lloyd, A. (1989). Bacteria associated with fruit flies and their host plants. *Fruit Flies, Their Biology, Natural Enemies and Control, 3*, 131–140.

Epsky, N., Heath, R., Dueben, B., Lauzon, C., Proveaux, A., & MacCollom, G. (1998). Attraction of 3-methyl-1-butanol and ammonia identified from *Enterobacter agglomerans* to *Anastrepha suspensa*. *Journal of Chemical Ecology, 24*, 1867–1880.

Husseneder, C. (2010). Symbiosis in subterranean termites: A review of insights from molecular studies. *Environmental Entomology, 39*, 378–388.

Jang, E. B., & Nishijima, K. A. (1990). Identification and attractancy of bacteria associated with *Dacus dorsalis* (Diptera: Tephritidae). *Environmental Entomology, 19*, 1726–1751.

Kai, M., Haustein, M., Molina, F., Petri, A., Scholz, B., & Piechulla, B. (2009). Bacterial volatiles and their action potential. *Applied Microbiology and Biotechnology, 81*, 1001–1012.

Kapoor, V. C. (2005). Taxonomy and biology of economically important fruit flies of India. *Israel Journal of Entomology, 35–36*, 459–475.

Kuzina, L. V., Peloquin, J. J., Vacek, D. C., & Miller, T. A. (2001). Isolation and identification of bacteria associated with adult laboratory Mexican fruit flies, *Anastrepha ludens* (Diptera: Tephritidae). *Current Microbiology, 42*, 290–294.

Lauzon, C. R., McCombs, S. D., Potter, S. E., & Peabody, N. C. (2009). Establishment and vertical passage of *Enterobacter* (*Pantoea*) *agglomerans* and *Klebsiella pneumoniae* through all life stages of the Mediterranean fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America, 102*, 85–95.

Lauzon, C. R., Sjogren, R. E., Wright, S. E., & Prokopy, R. J. (1998). Attraction of *Rhagoletis pomonella* (Diptera: Tephritidae) flies to odor of bacteria: Apparent confinement to specialized members of enterobacteriaceae. *Environmental Entomology, 27*, 853–857.

Lloyd, A., Drew, R. A. I., Teakle, D. S., & Hayward, A. (1986). Bacteria associated with some *Dacus* species (Diptera: Tephritidae) and their host fruit in Queensland. *Australian Journal of Biological Sciences, 39*, 361–368.

MacCollom, G., Lauzon, C., Sjogren, R., Meyer, W., & Olday, F. (2009). Association and attraction of blueberry maggot fly (Diptera: Tephritidae) to *Pantoea* (*Enterobacter*) *agglomerans. Environmental Entomology, 38*, 116–120.

Nakabachi, A., & Ishikawa, H. (1999). Provision of riboflavin to the host aphid, *Acyrthosiphon pisum*, by endosymbiotic bacteria, *Buchnera. Journal of Insect Physiology, 45*, 1–6.

Narit, T., & Anuchit, C. (2011). Attraction of *Bactrocera cucurbitae* and *B. papayae* (Diptera: Tephritidae) to the odor of the bacterium *Enterobacter cloacae. The Philippine Agricultural Scientist, 94*, 1–6.

Park, D. S., Oh, H., Jeong, W. N., Kim, H., Park, H. Y., & Bae, K. S. (2007). A culture-based study of the bacterial communities within the guts of nine longicorn beetle species and their exoenzyme producing properties for degrading xylan and pectin. *The Journal of Microbiology, 45*, 394–401.

Rinke, R., Costa, A. S., Fonseca, F. P. P., Almeida, L. C., Delalibera Junior, I., & Henrique-Silva, F. (2011). Microbial diversity in the larval gut of field and laboratory populations of the sugarcane weevil, *Sphenophorus levis* (Coleoptera, Curculionidae). *Genetics and Molecular Research, 10*, 2679–2691.

Robacker, D. C. (1991). Specific hunger in *Anastrepha ludens* (Diptera: Tephritidae): Effects on attractiveness of proteinaceous and fruit-derived lures. *Environmental Entomology, 20*, 1680–1686.

Robacker, D. C., & Bartelt, R. (1997). Chemicals attractive to Mexican fruit fly from *Klebsiella pneumoniae* and *Citrobacter freundii* cultures sampled by solid-phase microextraction. *Journal of Chemical Ecology, 23*, 2897–2915.

Robacker, D. C., & Flath, R. A. (1995). Attractants from *Staphylococcus aureus* cultures for Mexican fruit fly, *Anastrepha ludens. Journal of Chemical Ecology, 21*, 1861–1874.

Robacker, D. C., & Garcia, J. A. (1993). Effects of age, time of day, feeding history, and gamma irradiation on attraction of Mexican fruit flies (Diptera: Tephritidae), to bacterial odor in laboratory experiments. *Environmental Entomology, 22*, 1367–1374.

Robacker, D. C., Garcia, J. A., Martinez, A. J., & Kaufman, M. G. (1991). Strain of *Staphylococcus* attractive to laboratory strain *Anastrepha ludens* (Diptera: Tephritidae). *Annals of the Entomological Society of America, 84*, 555–559.

Robacker, D. C., Warfield, W. C., & Albach, R. F. (1993). Partial characterization and HPLC isolation of bacteria-produced attractants for the Mexican fruit fly, *Anastrepha ludens. Journal of Chemical Ecology, 19*, 543–557.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406–425.

Shi, Z., Wang, L., & Zhang, H. (2012). Low diversity bacterial community and the trapping activity of metabolites from cultivable bacteria species in the female reproductive system of the Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). *International Journal of Molecular Sciences, 13*, 6266–6278.

Sood, P., & Nath, A. (2005). Colonization of marker strains of bacteria in fruit fly, *Bactrocera tau. The Indian Journal of Agricultural Research, 39*, 103–109.

Sood, P., Prabhakar, C. S., & Nath, A. (2010). Ecofriendly management of fruit flies through their gut bacteria. *Journal of Insect Science, 23*, 275–283.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Thaochan, N., Drew, R. A. I., Hughes, J., Vijaysegaran, S., & Chinajariyawong, A. (2010). Alimentary tract bacteria isolated and identified with API-20E and molecular cloning techniques from Australian tropical fruit flies, *Bactrocera cacuminata* and *B. tryoni. Journal of Insect Science, 10*, 1–16.

Wang, H., Jin, L., Peng, T., Zhang, H., Chen, Q., & Hua, Y. (2013). Identification of cultivable bacteria in the intestinal tract of *Bactrocera dorsalis* from three different populations and determination of their attractive potential. *Pest Management Science*. doi:10.1002/ps.3528.

Wang, H., Jin, L., & Zhang, H. (2011). Comparison of the diversity of the bacterial communities in the intestinal tract of adult *Bactrocera dorsalis* from three different populations. *Journal of Applied Microbiology, 110*, 1390–1401.

Whitman, W. B., Goodfellow, M., Kämpfer, P., Busse, H. J., Trujillo, M. E., & Ludwig, W. (2012). *Bergey's manual of systematic bacteriology* (Vol. 5). New York, NY: Springer.