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Original Article

Gut bacterial community structure of two Australian tropical fruit fly species (Diptera: Tephritidae)

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Abstract

The community structure of the alimentary tract bacteria of two Australian fruit fly species, *Bactrocera cacuminata* (Hering) and *Bactrocera tryoni* (Froggatt), was studied using a molecular cloning method based on the 16S rRNA gene. Differences in the bacterial community structure were shown between the crops and midguts of the two species and sexes of each species. Proteobacteria was the dominant bacterial phylum in the flies, especially bacteria in the order Gammaproteobacteria which was prominent in all clones. The total bacterial community consisted of Proteobacteria (more than 75% of clones), except in the crop of *B. cacuminata* where more than 50% of clones belonged to Firmicutes. Firmicutes gave the number of the secondary community structure in the fly's gut. Four orders, Alpha-, Beta-, Delta- and Gammaproteobacteria and the phyla Firmicutes and Actinobacteria were found in both fruit fly species, while the order Epsilonproteobacteria and the phylum Bacteroidetes were found only in *B. tryoni.* Two phyla, Actinobacteria and Bacteroidetes, were rare and less frequent in the flies. There was a greater diversity of bacteria in the crop of the two fruit fly species than in the midgut. The midgut of *B. tryoni* females and the midgut of *B. cacuminata* males had the lowest bacterial diversity.

Keywords: *Bactrocera cacuminata, Bactrocera tryoni,* gut bacteria community, 16SrRNA gene, molecular cloning

1. Introduction

Previous studies on bacteria associated with the alimentary tract of adult fruit flies (Diptera: Tephritidae) have focused on species in the genera *Enterobacter, Klebsiella, Citrobacter, Erwinia, Escherichia, Pantoea, Pseudomonas* and *Serratia* (family Enterobacteriaceae, phylum Proteobacteria). These were bacteria species culturable on artificial medium and found to be dominant in the fruit fly alimentary canal (Drew & Lloyd, 1987, 1989, 1991; Fitt & O'Brien, 1985; Lloyd *et al.,* 1986; Kuzina *et al.,* 2001; Marchini *et al.*, 2002). Although these genera appear to be common in Tephritidae around the world, no single bacterial species occurs consistently within one fruit fly species. The presence of a relatively small number of bacteria species in adult flies, on oviposition sites and in developing larvae has, in previous reports, been suggested as an indication of a close specific association between fruit flies and these microorganisms (Drew *et al.*, 1983; Drew & Lloyd, 1987, 1989, 1991; Drew & Romig, 1999). The bacteria were generally believed to be mutualistic

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symbionts of the flies (Drew & Lloyd, 1991; Drew and Romig, 1999; Capuzzo *et al.*, 2005). However, other bacteria, including less common species, were overlooked because they did not grow on, or could not compete with, the fast growth rates of Enterobacteriaceae species on the chosen nutrient agar media (Thaochan *et al.*, 2010a, b).

Molecular techniques have been used to study gut bacteria in insects and have revealed more taxa and larger microbe community structures, including both rare species and unculturable microorganisms (Haynes *et al.*, 2003; Egert *et al.*, 2005; Schmitt-Wagner *et al.*, 2003; Broderick *et al.*, 2004; Nakajima *et al.*, 2005; Cox & Gilmore, 2007; Miyata *et al.*, 2007). These techniques have provided a more holistic diagnosis of the bacterial community structures in insects (Amann *et al.*, 1995).

In general, the fruit fly alimentary tract consists of three main parts where bacteria reside, the oesophageal bulb, crop and midgut (Drew *et al.*, 1983). Each part performs different physiological functions and has different pH conditions (Drew *et al.*, 1983; Fitt & O'Brien, 1985). In other insects studied, the bacterial community differs between the different parts of the alimentary tract, both in community structure and relative abundance of species (Friedrich *et al.*, 2001; Nakajima *et al.*, 2005; Schmitt-Wagner *et al.*, 2003; Egert *et al.*, 2005). This paper presents results from applying the 16S rRNA gene molecular cloning technique to a study of the gut bacteria community structure in two Australian fruit fly species, *Bactrocera cacuminata* (Hering) and *Bactrocera tryoni* (Froggatt). Data were analysed to compare the community similarity, community structure, relative abundance, species richness and diversity of gut bacteria between the crop and midgut and between males and females of both fruit fly species.

2. Materials and Methods

2.1 Fruit fly collecting and handling

Males and females of *B. cacuminata* and *B. tryoni* were hand collected from fruiting host plants in Brisbane, Queensland, Australia, during the months of February and March, 2007. *B. cacuminata* were collected from wild tobacco (Solanum *mauritianum* Scopoli) and *B. tryoni* were collected from custard apple (*Annona reticulata* L.), guava (*Psidium guajava* L.) and loquat (*Eriobotrya japonica* (Thunb.) Lindl.). Each fly was held separately in a small clear, clean plastic vial to prevent cross-contamination of bacteria between flies. In the field, the vials containing flies were closed with cotton wool for ventilation and placed in a cool ice box for transport to the laboratory.

2.2 Fruit fly dissection

Five males and five females of each species were prepared for dissection immediately on return to the laboratory, Griffith University, Brisbane, as follows – killed by freezing at -20°C for 3 min, surface sterilized by immersing in 70% ethanol for 1 min, 0.5% sodiumhypochlorite for 1 min and washed twice in sterile distilled water (modified from Lloyd, 1991). Surface-sterilized flies were then individually dissected under sterile distilled water in a sterile glass cavity block. The crop and midgut were removed and placed into separate sterile 1.5 ml centrifuge tubes for DNA extraction.

2.3 DNA extraction and PCR

DNA was extracted using a modification of the CTAB/phenol-chloroform DNA extraction protocol (Doyle & Doyle, 1987). DNA from individual samples was amplified in a polymerase chain reaction (PCR) using the universal primers for bacteria with forward primer Y1 (5′-TGGCTCAGAACGAACGCTGGCGGC-3′) (Sigma) and reverse primer Y2 (5′-CCCACTGCTGCCTCCCGTAGGAGT-3′) (Sigma) (Young *et al.*, 1991). The reactions were carried out in a 100 µl volume containing 2 µl of template DNA solution, 2 µM of primer, 200 µM of deoxynucleosidetriphosphate (Astral scientific, Bioline) and 2 U of Tag DNA polymerase (Astral scientific, Bioline). The amplifications were performed using the following protocol: initial denaturation at 94°C for 5 min; 30 cycles of 45 s at 94°C, 40 s at 62°C and 2 min at 72°C, and final extension at 72°C for 10 min. After the reaction, 5 µl aliquots of PCR products were examined by electrophoresis in 1% agarose gel and the target DNA extracted (500-700 bp) with the QIAquick gel extraction kit (Qiagen).

2.4 Molecular cloning

The ligations were performed in 10 µl solution containing 1 µl pDrive cloning vector (50 ng/µl), 2.5 µl mix DNA, 1.5 µl distilled water, and 5 µl 2x ligation master mix (Qiagen) and introduced into *Escherichia coli* (strain JM109, Promega) by transformation. The recombinants were selected and verified to be the correct insert size by vector-targeted PCR with primer M13 F (5′-GTAAAACGACGGCCAGT-3′) (Sigma) and M13 R (5′-CAGGAAACAGCTATGAC-3′) (Sigma) by the following PCR protocol: an initial denaturation at 94°C for 4 min; 35 cycles of 30 sec at 94°C, 40 sec at 53°C and 60 sec at 72°C. Finally, samples were subjected to 72°C for 4 min and then held for an indefinite period at 4°C. From each clone library, 30 clones were randomly selected and sequenced. Sequences were determined on an 3130xl Genetic Analyser (Applied Biosystems) at the Griffith University DNA Sequencing Facility.

2.5 Estimation of microbial diversity

Diversity coverages were estimated from the clone libraries for each sample site, using the Analytical Rarefaction software (version 1.2; S. M. Holland, University of Georgia, Athens, Ga; http://www.uga.edu/strata/Software.html). The species richness, diversity (Shannon & Weaver, 1963) and evenness (Margalef, 1958) of each clone library, were

Table 1. Number of clones from two fruit flies species, *Bactrocera cacuminata* (Hering) and *B. tryoni* (Froggatt), with sequence similarity 75-100%.

Bacterial taxa	*B. cacuminata*		Total	*B. tryoni*		Total
	Male	Female		Male	Female	
Alphaproteobacteria	5	10	15	9	27	36
Betaproteobacteria	4	1	5	5	11	16
Deltaproteobacteria	19	0	19	12	1	13
Epsilonproteobacteria	0	0	0	1	0	1
Gammaproteobacteria	37	52	89	56	47	103
Actinobacteria	2	2	4	1	2	3
Bacteroidetes	0	0	0	2	0	2
Firmicutes	33	35	68	14	12	26
Total	100	100	200	100	100	200

analyzed with the Shannon-Weaver index, for microbes with a sequence similarity of 97%. The Shannon-Weaver index of diversity (*H'*) was calculated using the formula $H' = -\Sigma\, pi\, (\ln pi)$, where *pi* is the abundance of phylotypes calculated from *n/S, n* is the number of phylotypes presented in each clone library and *S* (species richness) the total number of sequences with 97% sequence similarity of bacteria species in the data set. The Shannon-Weaver index of evenness (E_H) was calculated using the formula $E_H = H'/Hmax$ and $Hmax = \ln S$. Equitability assumes a value between 0 and 1, with 1 being complete evenness (Shannon & Weaver, 1963). The similarities of phylotype profiles for each clone library were analyzed using Primer software (Primer 5, United Kingdom) as described by Rooney *et al.* (2006). Briefly, non-metric multidimensional scaling (MDS) plots were used to create and visualize the community similarity structure of each clone library. The MDS data estimated the similarity distance between any two communities from the points on the plot (Kruskal, 1964). The higher community similarities are represented by points closest together, while the lower similarities are represented by points located further apart. The degree of similarity matrix was judged by examining stress (Kruskal's stress), with values of less than 0.1 representing good ordinations with little risk of misinterpretation of the data (Clarke, 1993). The MDS plots were used to visualize the relationships between bacteria communities growing in the different parts of the alimentary tract (crop and midgut) and within each sex of the two fruit fly species.

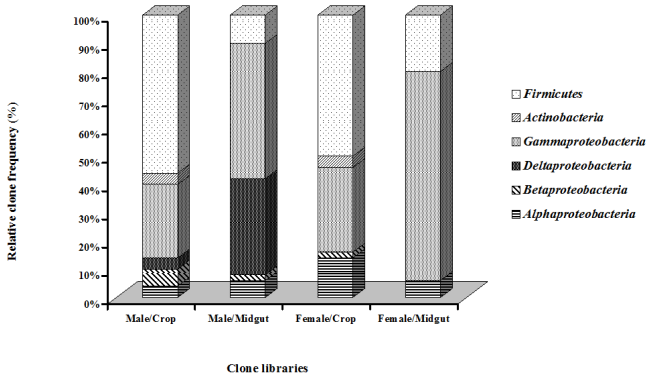

Figure 1. Relative clone frequency (%) of the clone libraries of the four locations of gut bacteria community of *Bactrocera cacuminata* (Hering) between sexes (male and female) and alimentary tract site (crop and midgut). Data of 200 clones from the gut bacteria community were used for comparison. All clones had percentage similarity 75-100%.

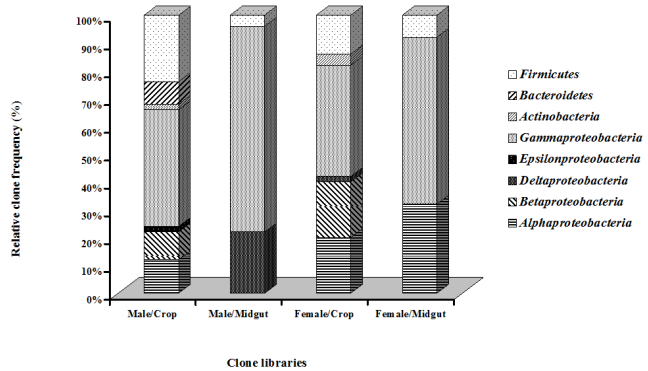

Figure 2. Relative clone frequency (%) of the clone libraries of the four locations of gut bacteria community of *Bactrocera tryoni* (Froggatt) between sexes (male and female) and alimentary tract site (crop and midgut). Data of 200 clones from the gut bacteria community were used for comparison. All clones had percentage similarity 75-100%.

3. Results

3.1 Bacteria taxa isolated

A total of 400 clones with an 80-100% sequence similarity were isolated from the crop and midgut of *B. cacuminata* and *B. tryoni* (Table 1). The species of gut bacteria diagnosed in either or both *B. cacuminata* (Figure 1) and *B. tryoni* (Figure 2) possessed similarities to known

species of gut bacteria. Six bacterial taxa, Actinobacteria, Firmicutes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria were identified. Alpha-, Beta-, Delta- and Gammaproteobacteria were the most abundant (85.63%, 149 clones) in all clone libraries. Species within the order Gammaproteobacteria were represented by 99 clones (56.89%), then Alpha- 26 clones (14.94%), Beta- 16 clones (9.19%) and Delta- 8 clones (4.59%). The most abundant phylotypes were found in the order Gammaproteobacteria with *Serratia* sp., *Enterobacter* sp., *Klebsiella* sp. and *Escherichia coli* comprising 18 clones (10.34%), one clone (6.32%), 11 clones (6.32%) and 10 clones (5.75%), respectively. Eleven clones (6.32%) of *Ochrobactrum* were the most abundant phylotypes in the order Alphaproteobacteria. Five clones (2.91%) of *Ralstonia detusculanense* and five clones (2.91%) of *Variovorax* sp. were predominant in the order Betaproteobacteria. In Deltaproteobacteria, only one phylotype, *Desulfovibrio* sp., with eight clones (4.59%) was found. Firmicutes were represented by 21 clones (12.07%) with the most abundant phylotypes being *Leuconostoc fructosus* with seven clones (4.02%) and *Lactobacillus fructivorans* with five clones (2.87%). Actinobacteria were less frequent with four clones (2.29%) and represented by *Micrococcus luteus* with one clone (0.57%), *Propionibacterium acnes* with one clone (0.57%) and *Corynebacterium* sp. with two clones (1.15%).

3.2 Bacteria isolated from *Bactrocera cacuminata*

Bactrocera cacuminata females contained five bacteria taxa, Actinobacteria, Firmicutes, Alpha-, Beta- and Gammaproteobacteria (Figure 1), with 63% of the clones belonging to Proteobacteria, which was represented by Gammaproteobacteria (52%), Alphaproteobacteria (10%) and Betaproteobacteria (1%) (Table 1). Thirty five percent of the clones belonged to Firmicutes and 2% to Actinobacteria. Six taxa were isolated from males of *B. cacuminata.* These were the five found in the females plus Deltaproteobacteria. Sixty five percent of the clones isolated from male *B. cacuminata* were Proteobacteria of which Gammaproteobacteria (37%), Deltaproteobacteria (19%), Alphaproteobacteria (5%) and Betaproteobacteria (4%) were most common. Firmicutes and Actinobacteria were represented by 33% and 2%, of the clones respectively.

3.3 Bacteria isolated from *Bactrocera tryoni*

Six bacterial taxa were found in the alimentary tract of female *B. tryoni* (Table 1), the same as in males of *B. cacuminata.* The Proteobacteria were the most abundant (86%), comprised primarily of Gammaproteobacteria (47%), Alphaproteobacteria (27%), Betaproteobacteria (11%) and Deltaproteobacteria (1%). Firmicutes were represented by 12% and Actinobacteria 2% of clones. Eight taxa were found in males of *B. tryoni,* the six found in females, plus Epsilonproteobacteria and Bacteroidetes. The Proteobacteria (83%) were the most abundant, consisting of Gammaproteobacteria (56%), Deltaproteobacteria (12%), Alphaproteobacteria (9%), Betaproteobacteria (5%) and Epsilonproteobacteria (1%). The other taxa, Firmicutes, Actinobacteria and Bacteroidetes were found at the rate of 14%, 1% and 2%, respectively (Figure 2).

3.4 Biodiversity of fruit fly gut bacteria

More bacteria taxa were found in the crop of male *B. tryoni* (seven taxa) and fewer in the midgut of female *B. cacuminata* (three taxa) (Figure 1 and 2). The midgut of female and male of *B. tryoni* possessed three taxa from each clone library. The Gammaproteobacteria and Firmicutes were dominant in all clone libraries. The total number of clones, number of phylotypes, species richness, Shannon-Weaver index (Shannon and Weaver, 1963) and evenness (Margalef, 1958) of the bacterial communities are presented in Table 2. Each library had 50 clones per sample site. The crop of female *B. tryoni* and midgut of male *B. cacuminata* possessed the highest (20) and lowest (7) species richness, respectively. The diversity of species, as expressed though the Shannon-Weaver index and Evenness values, was higher for the crop of female *B. tryoni* (SWI 2.7) and lowest for the midgut of male *B. cacuminata* (SWI 1.4). However, the Evenness values were highest for the crop of male *B. cacuminata* (0.96) and lowest for the midgut of male *B. cacuminata* (0.72). The diversity coverage by each clone library is presented in the rarefaction curves (Figure 3 and 4). In all clone libraries, the crop of both species demonstrated higher diversity than the midgut. The highest diversity was found in the crop of female *B. tryoni* (Figure 4) and lowest in the midgut of male *B. cacuminata* (Figure 3).

3.5 Multidimensional scaling analysis (MDS)

The non-metric multidimensional scaling (MDS) analyses, based on the 16S rRNA gene of each bacteria clone, revealed that the similarity values were more than 97% on the NCBI database (Figure 5 and 6). In comparing 174 phylotypes at the species level (>97% sequence similarity) the crop and midgut of *B. cacuminata* (Figure 5) and *B. tryoni* (Figure 6) shared some bacterial species, while males and females exhibited considerable differences.

4. Discussion

In this study, the two fruit fly species, *B. cacuminata* and *B. tryoni,* possessed distinct gut bacteria communities. Firmicutes and Proteobacteria were present in all clone libraries (Figure 1 and 2) and were also the most abundant. The recording of Proteobacteria was similar to results reported for *B. tryoni* (Drew & Lloyd, 1989, 1991), *Anastrepha ludens* (Kuzina *et al.,* 2001) and *Ceratitis capitata* (Marchini *et al.*, 2002). In particular, Gammaproteobacteria was the dominant order, represented by the genera *Entero-*

Table 2. Phylotype richness, diversity and evenness index of bacterial community in difference of fruit flies species, *Bactrocera cacuminata* (Hering) and *B. tryoni* (Froggatt), sexes and alimentary tract.

Fruit flies	Sexes	Site	No. of clones	No. of phylotypes [a]	Species richness [b]	Shannon-Weaver index [c]	Evenness [d]
B. cacuminata	Female	Crop	50	18	10	1.99	0.86
		Midgut	50	18	9	2.06	0.94
	Male	Crop	50	14	9	2.11	0.96
		Midgut	50	22	7	1.40	0.72
B. tryoni	Female	Crop	50	37	20	2.70	0.90
		Midgut	50	18	7	1.54	0.79
	Male	Crop	50	22	12	2.26	0.91
		Midgut	50	25	10	1.94	0.84

[a] Total number of phylotypes based on 97% sequence identity.
[b] Species richness (*S*)
[c] Shannon-Weaver index (*H'*)
[d] Evenness (E=$H'/H_{max,}$ H_{max}= ln*S*)

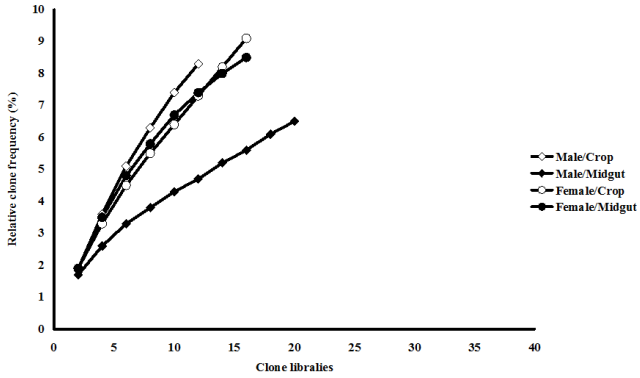

Figure 3. Rarefaction analysis of all clones libraries recovered *Bactrocera cacuminata* (Hering) comparing between sexes (male and female) and alimentary tract sites (crop and midgut). Rarefaction curves represent 97% sequence similarity.

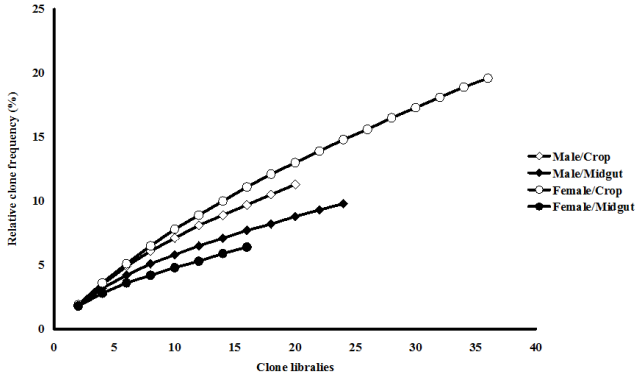

Figure 4. Rarefaction analysis of all clones libraries recovered *Bactrocera tryoni* (Froggatt) comparing between sexes (male and female) and alimentary tract sites (crop and midgut). Rarefaction curves represent 97% sequence similarity.

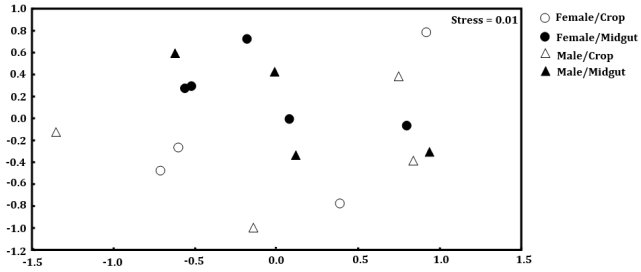

Figure 5. Multidimensional scaling of bacterial community similarity at species level (>97% sequence similarity) between crop and midgut of *Bactrocera cacuminata* (Hering).

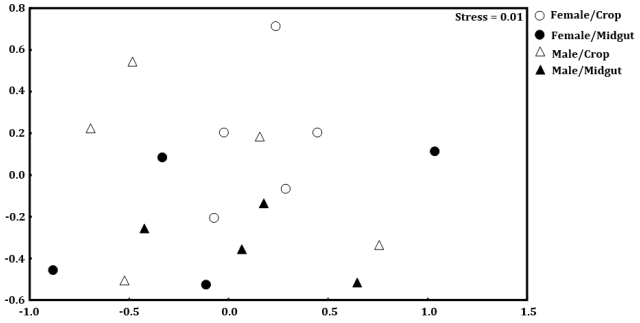

Figure 6. Multidimensional scaling of bacterial community similarity at species level (>97% sequence similarity) between crop and midgut of *Bactrocera tryoni* (Froggatt).

bacter, Klebsiella, Citrobacter, Serratia, Pantoea and *Pseudomonas*. In addition, the genera *Leuconostoc* and *Lactobacillus* within Firmicutes, were also present. These genera have not previously been reported in any fruit fly species. Recent molecular studies, based on the 16S rRNA gene, have revealed that similar gut bacteria species have been discovered in other insects. Some examples are the African higher termite, *Nasutitermes lujae* (Paster *et al.*,

1996), the pomace fly, *Drosophila melanogaster* (Cox & Gilmore, 2007), the Western flower thrips, *Frankliniella occidentalis* (de Vries *et al.*, 2001), the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae) (Egert *et al.*, 2003), the pea aphid *Acyrthosiphon pisum* and the black bean aphid *Aphis fabae* (Haynes *et al.*, 2003).

For *B. cacuminata,* the dominant phyla in the crop belonged to Firmicutes while those in the midgut were species of Proteobacteria. In contrast, for *B. tryoni,* Proteobacteria species were dominant in both the crop and midgut. These results were similar to those reported for *D. melanogaster* (Cox & Gilmore, 2007), the gypsy moth *Lymantria dispar* L. (Broderick *et al.*, 2004), the southern pine beetle *Dendroctonus frontalis* Zimmermann (Vasanthakumar *et al.*, 2006) and the soil-feeding termite *Cubitermes niokoloensis* (Fall *et al.*, 2007).

The biodiversity of alimentary tract bacteria, expressed through the Shannon-Weaver Index, was greater in the crop than the midgut for both *B. cacuminata* and *B. tryoni,* except for female *B. cacuminata*, where the midgut contents were more diverse than those in the crop (Table 2). The Evenness Value, based on species diagnosed through the 16S rRNA gene with a similarity to known database species of 97%, demonstrated that all alimentary tract sites had a high degree of equitability (>0.72) (Table 2). Further, the rarefaction curves were steeper for the crop than the midgut, for both fruit fly species (Figure 3 and 4), demonstrating that the crop microbial contents were more diverse. These rarefaction curve results were in conformity with the Shannon-Weaver index (Figure 3, 4 and Table 2). In other insect species e.g. the termite, *Reticulitermes speratus,* the clones recovered from the gut wall had steeper rarefaction curves and thus greater diversity than the gut lumen, indicating that colonization of bacteria was higher in gut wall. This pattern was thought to be advantageous for the retainment of symbionts in the gut, holding them in the wall to prevent being moved out by the flow of food. Also, the gut wall was considered a site for oxygen penetration for uptake by the bacteria (Brune & Friedrich, 2000; Nagajima *et al.*, 2005).

At least 40% of all clones were Proteobacteria and this high incidence is similar to that recorded in *D. melanogaster* (Cox & Gilmore, 2007) where a 61% recovery was measured. Within this phylum, the Gammaproteobacteria (49.25% incidence) was the principal group in both *Bactrocera* species, which was again similar to results in *D. melanogaster* which recorded a 43.6% incidence of bacteria species in this order (Cox & Gilmore, 2007). Altogether, five orders within the phylum Proteobacteria were recorded from these fruit fly species, in contrast to the gypsy moth which contained primarily one, the Gammaproteobacteria (Broderick *et al.*, 2004). Also, in contrast to *D. melanogaster,* the genus *Wolbachia* was not recorded in the *Bactrocera* species. This is a group of species that can be transmitted between various arthropods, including many insect species, and are recognized as important pathogens which may be useful in biological control of pest insects (Sasaki & Ishikawa, 1998).

The gut bacteria community structure in this study was comparable to that previously recorded in *B. cacuminata* and *B. tryoni* (Drew *et al.*, 1983) and *D. melanogaster* (Cox & Gilmore, 2007). Also, there has been reported a very similar bacteria community in the alimentary tract of termites, although these insects have a higher level of biodiversity than do *Bactrocera* species (Fall *et al.*, 2007; Schmitt-Wagner *et al.*, 2003). In gypsy moth larvae, 70% of all bacteria are Proteobacteria with *Serratia marcescens* the dominant species (Broderick *et al.*, 2004). The scarab beetle larvae, *Melolontha melolontha,* also possess a rich microflora of Proteobacteria (Egert *et al.*, 2005) while the termite, *Nasutitermes takasagoensis,* possesses fewer bacteria in this phylum (Brune *et al.,* 1995; Brune & Kuhl, 1996). It is possible that the different food sources for insects have an influence on the biodiversity of the alimentary tract microflora.

The different parts of the alimentary tract possessed a different biodiversity of bacteria colonies. In the crop of *B. cacuminata,* 53% of the clones belonged to Firmicutes whereas the midgut contained only 15% of this phylum.

In *B. cacuminata,* species of lactic acid bacteria identified were *Leuconostoc fructosus* (31.82% of total Firmicutes clones), *Lactobacillus fructivorans* (13.63%) and *Lactobacillus sanfranciscensis* (9.09%) while in *B. tryoni* gut bacteria *L. fructivorans* (9.09%) was the only species of Firmicutes diagnosed.

The phyla of lower incidence in the *Bactrocera* species were Actinobacteria (1.75% of all clones) and Bacteroidetes (1%), with the latter only found in the crop of *B. tryoni.* This diversity is very similar to that reported for *D. melanogaster* (Cox & Gilmore, 2007). In contrast, species in Bacteroidetes were the most abundant group in termites (Miyata *et al.*, 2007; Ohkuma *et al.*, 2002; Noda *et al.*, 2005) where they have been reported as important in the metabolism of plant fibres and other dietary components (Avgustin *et al.*, 1997).

The physiological environment of the different parts of the insect alimentary canal appears to have an influence on the bacteria species that are present (Lemke *et al.*, 2003). For example, the pH of the crop of *Bactrocera* spp. is 3.0-3.5 (Drew *et al.*, 1983) and a dominant group of bacteria in this organ belong to the acid metabolising genera *Leuconostoc* and *Lactobacillus.* Similarly, the midgut pH is slightly alkaline, 5.7-7.4 and the most common bacteria belong to the genera *Citrobacter, Enterobacter, Klebsiella* and *Pantoea,* all of which prefer a neutral to alkaline environment.

It is also possible that the diversity of bacteria in the alimentary canal of insects is partly related to the respective diets. The bacteria communities in *D. melanogaster,* the larvae of the gypsy moth and scarab beetle, that feed on fresh plant material, are more similar to one another and markedly different from termites that are soil dwelling and feeding on dry plant material.

The application of molecular techniques in the study of alimentary tract microorganisms in *Bactrocera* species, has provided a more complete understanding of their micro-

bial biodiversity. A large range of bacteria were diagnosed that were not isolated and identified through the standard culture dependent techniques (Thaochan *et al.*, 2010a, b). This research should be expanded to include more tephritid species and also to investigate the ecological relationships between the flies, their host fruits and bacteria, particularly those in the phylum Firmicutes that are known to occur on ripe fruit surfaces (Antunes *et al.*, 2002).

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