

Assessing the effects of gut bacteria manipulation on the development of the oriental fruit fly, *Bactrocera dorsalis* (Diptera; Tephritidae)

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Abstract *Bactrocera dorsalis* (Diptera: Tephritidae) is a serious menace to agricultural production worldwide. In order to prevent further damage, it is of paramount important that cost-effective strategies should be developed for their management. Gut bacteria has established diverse relationships with their insect hosts, which could be exploited in pest management programs to improve on control efficiency. In this study, gut bacteria isolates identified by culture dependent technique were incorporated into larval diets in an attempt to understand the roles they play in the development and survival of oriental fruit fly. From our results, the isolated bacteria belonged to four different phyla including the Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria. The response of the fly to different gut isolates varied greatly. Diets enriched with *Enterococcus phoeniculicola* had lower larval developmental duration, higher pupal weight, and an increased percentage survival. On the other hand, diets supplemented with *Lactobacillus lactis* had negative effects on *B. dorsalis* development. This study provides clues on how symbiotic bacteria could be exploited in mass rearing for an efficient implementation of the Sterile Insect Technique (SIT) in pest management programs.

Keywords *Bactrocera dorsalis* · Gut bacteria · Fruit fly · Fitness · Mass rearing

1 Introduction

The Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera; Tephritidae) is one of the most important agricultural pests with over 350 hosts worldwide (Drew 2004; Clarke et al. 2005). In recent years, the management of the Oriental fruit fly has attracted a lot of attention due to the high levels of economic losses caused by this pest (Caceres et al. 2014; Schutze et al. 2015). Therefore different control strategies are currently being used in the management of this pest (Vargas et al. 2007; Bhagat et al. 2013). One of such techniques is the Sterile Insect Technique (SIT) which has been successfully incorporated into integrated pest management programs for the control of the tephritids (Orankanok et al. 2007; Barclay et al. 2014; Vargas et al. 2015). A typical example is the successful eradication of *B. dorsalis* in Mariana Islands (Steiner et al. 1970). In SIT programs, production of sterile males in large quantities is of paramount importance and very expensive. It is also crucial for these laboratory-reared flies to possess some fitness attributes that could enable them to survive and out-compete wild flies after their release (Hamden et al. 2013). Therefore, there is an urgent need for research to be carried out to develop cost-effective production methods and improve on the copulatory success of laboratory-reared flies.

Though the associations of tephritids and their symbionts have been known for almost a century (Petri 1910) it was only much later that these symbionts were first reported to play a role in the biology of these flies (Hagen 1966). In an attempt to understand the roles symbiotic bacteria play in symbiotic relationships with these flies, extensive studies have been

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conducted with the aid of antibiotics and microbial dietary supplements often referred to as probiotics (Ben-Ami et al. 2010; Ben-Yosef et al. 2010; Gavriel et al. 2011). From these studies symbiotic bacteria has been reported to play diverse roles in their relationship with fruit flies. For example, *Klebsiella oxytoca* has been shown to improve mating competitiveness of male flies (Behar et al. 2005), *Candidatus Erwinia dacicola* contributes essential amino acid (Ben-Yosef et al. 2014) and enable the insect to overcome host defense (Ben-Yosef et al. 2015), *Bacillus cereus* has been shown to act as an attractant lure for *B. dorsalis* (Wang et al. 2014a), *Citrobacter freundii* has been reported to break down insecticides leading to insecticide resistance (Cheng et al. 2017) and *Wolbachia* was found to reduce female fecundity and adult lifespan in Medfly, *Ceratitis capitata* (Sarakatsanou et al. 2011). Although a lot of work has been carried out on insect microbial interactions, there are still some unsolved questions that need to be addressed. For example, for given fruit fly species, what specific roles do individual microbial species play when present in the gut of the insect? Do these roles have a huge impact on the insect's fitness and survival?

To the best of our knowledge, only a few studies have focused on the impact that individual microbial species have on fruit fly fitness (Gavriel et al. 2011; Ben-Ami et al. 2010). Furthermore, most of the gut isolates used for those studies were Enterobacteriaceae (Hamden et al. 2013; Yuval et al. 2013; Augustinos et al. 2015). This is because Enterobacteriaceae has been reported to be the most abundant family in many Tephritids including Medfly *Ceratitis capitata* (Aharon et al. 2013), Chinese citrus fly *Bactrocera minax* (Wang et al. 2014b). However, other studies has reported the Enterococaceae to be one of the dominant groups in fruit flies (Andongma et al. 2015; Morrow et al. 2015). Thus in the present study, *Bactrocera dorsalis* genetic sexing strain (GSS) was used as model insects to understand the effects of supplementing larval diets with different bacteria isolates. The objective of this study was to screen probiotics that could be used to empower the Sterile Insect Technique (SIT) in the management of fruit fly pests in China and around the world.

2 Material and methods

2.1 The insect strain and rearing conditions

The experiments were carried out using *B. dorsalis* GSS previously obtained from laboratory stocks at the Insect Pest Control Laboratory of International Atomic Energy Agency (IAEA), Seibersdorf, Austria, and currently being reared at the College of Plant Science and Technology, Huazhong Agricultural University. Adult flies were maintained in mesh cages on an artificial diet of tryptone, yeast extract and sugar in the proportion of 1: 2: 4. The rearing conditions were under

temperature of 27 ± 2 °C, 70–80% relative humidity and photoperiod of 14:10 L:D.

2.2 Isolation of gut bacteria

Adult gut bacteria was isolated by culture dependent technique as follows: 15 flies were collected and anesthetized by exposing them to -20 °C for 10 min. Flies were then surface sterilized in 10% Tween for 1 min, followed an immersion in 0.2% sodium hypochlorite for 1 min, then 70% ethanol for 2 min and finally rinsed twice in sterile distilled water. Gut dissection was carried out aseptically under a light stereomicroscope in a laminar flow hood. The midgut was carefully dissected and placed in tubes containing 700 μ l of phosphate buffer saline (pH 7). Homogenized gut suspensions were serially diluted and plated on a Luria Bertini (LB) agar media. The plates were incubated at 37 °C for 24–48 h. Fifty distinct colonies were selected based on their morphological characteristics and purified by replating thrice on LB plates prior to DNA extraction.

2.3 Bacteria DNA extraction

Before DNA extraction, bacteria stock solution was plated on LB, then a single colony was cultured in LB broth for about 7 h. Bacterial suspensions in LB broth were harvested by centrifugation and the bacterial pellets were re-suspended in 557 μ l of TE buffer (10 mmol/ Tris-HCl, 50 mmol/l EDTA). Ten micro litres of lysozyme (5 mg/ml) was added to the suspension and the reaction incubated for 20 min at 37 °C. Then, 30 μ l SDS (10%) and 3 μ l proteinases K (20 mg/ml) was added and incubated again at 37 °C for 40 mins. Finally, 100 μ l of NaCl (5 mol/l) and 80 μ l of CTAB/NaCl was added followed by an incubation for 10 mins at 65 °C. Extraction of the DNA samples were carried out using phenol/chloroform/isoamyl alcohol (25:24:1) and later centrifuged at 13400 g for 4 min. DNA pellets were precipitated with isopropyl alcohol, rinsed in 70% ethanol, and finally resuspended in TE buffer.

2.4 PCR amplification

PCR amplification of the bacterial 16S rRNA gene was carried out using the bacterial universal primers 27F:5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-GGTTACCTTGTTACGACTT-3'. The final PCR reaction buffer of 25 μ l contained 0.2 mM each of forward and reverse primers, 1 \times PCR reaction buffer, ~50 ng of template DNA and 1 U of *Pfu* DNA Polymerase (MBI. Fermentas, USA). PCR condition were: an initial denaturation step of 95 °C for 5 min followed by 34 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, an extension phase of 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on a 1% agarose

gel and visualized under UV light after staining with ethidium bromide. The target band of 1500 bp was purified with a DNA gel extraction kit (Axygen, China). The DNA sequences were analyzed at Novogene (China) and sequence identity were known after a blast on NCBI nucleotide collection (nr/nt) using the megablast algorithm (<http://blast.ncbi.nlm.nih.gov/>).

2.5 Preparation of experimental larval diet and feeding

The gut bacteria isolates were cultured individually in LB broth and harvested by centrifugation. They were later washed twice with sterile distilled water and resuspended again in sterile distilled water. The concentration of bacteria in solvent was adjusted to an OD of 0.4 at 550 nm with the aid of a spectrophotometer (Eppendorf AG, Germany). Five hundred microliters of bacteria suspension was added to 50 g diets consisting of wheat bran, yeast extract and sugar in the proportions 4: 1: 2, which had previously been autoclaved and cooled to room temperature. Similarly, 500 μ l of sterile distilled water was added to autoclaved diets and used as a control. Petri dishes containing larval diet were sealed and incubated overnight at 37 °C. A total of 200 eggs were carefully selected under light microscope and placed on wet filter paper. The filter papers (with eggs) were later transferred to petri dishes containing diet (Augustinos et al. 2015). Each petri dishes were placed in a boxes containing sterile sand and incubated under the following conditions: temperature 27 ± 2 °C, 70–80% relative humidity and photoperiod of 14:10 L: D. Each treatment replicated thrice.

2.6 The effect of bacteria on developmental duration

The number of larvae that pupated each day were counted. This allowed for the estimation of the larval developmental time. Similarly, daily cohort of pupae were kept separately in tubes and checked daily for adult emergence, this allowed for the estimation of the pupal developmental time. The total developmental time was estimated by adding the larval and pupal developmental durations. For each experiment, partially emerged insects were counted as dead.

2.7 The effect of bacteria on insect survival

In order to estimate the proportion of insects that survived each developmental stage, the number of insects present at the end of the each life stages were counted. The proportion of surviving larvae was estimated by dividing the pupal number by the original number of eggs. Similarly, the proportion of surviving pupae were estimated by dividing the total number of emerged adults by the pupal number.

2.8 The effect of gut bacterial isolates on pupal weight

In order to determine the mean pupal weight per treatment, pupal weight was estimated by group weighing 7 days old pupae. Pupal measurements were carried out as follows: for pupation days with more than five pupae per replicate, five pupae were group weighed in batches of five, for pupation days with less than five pupae per replicate. All the pupae were weighed together.

2.9 Statistical analysis

Data analyses were performed using SPSS (SPSS Inc., Chicago, IL, U.S.A.). All data are presented as mean \pm Standard Error (SE). Differences between groups were tested using one-way analysis of variance (ANOVA) to infer the effects of the probiotic provision on pupal weight, developmental duration and survival. Significant differences between treatments were determined by Post hoc using LSD at the 0.05 level. The Pearson's correlation analysis was used to determine the interaction between average weight and other parameters (development time and survival rate) at a 0.05 significant level.

3 Results

3.1 Gut bacterial community

A Blastn search showed that the bacteria stains isolated from the gut of the *B. dorsalis* GSS related to 13 different bacteria species which could be assigned to Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria (Table 1). Out of these, eight were Firmicutes including *Enterococcus gallinarum*, *E. phoeniculicola*, *E. avium*, *E. termitis*, *Lysinibacillus macrolides*, *Lactococcus garvieae*, *L. lactis* and *Vagococcus fluvialis*. The Proteobacteria was represented by three strains relating to *Klebsiella pneumoniae*, *Morganella morganii* and *Citrobacter freundii*. The other two strains relating to the bacteria *Myroides odoratimimus* and *Microbacterium* sp. belonged to the Bacteroidetes and Actinobacteria, respectively.

3.2 Effect of bacteria on developmental duration

Larval developmental duration The larval diet supplemented with bacteria of all *Enterococcus* sp. relatively reduced the pre-pupal developmental duration when compared with the control treatment (CK) (Fig. 1a). However this effect was significant in *E. phoeniculicola* (Ep) (8.29 days) and *E. avium* (Ea) (8.41 days) supplemented diets ($P = 0.011$ and $P = 0.046$, respectively). Although larvae reared on *Microbacterium* sp. (Mic), *K. pneumoniae* (Kp) and *V. fluvialis* (Vf) treatments had a significantly longer larval

Table 1 Identification of the bacterial groups isolated from the gut of the oriental fruit fly, *Bactrocera dorsalis* based on the 16S rRNA gene sequencing

Isolated	Family	Closest matches	Accession No.	% Identity
Bd-1	Enterococcaceae	<i>Enterococcus gallinarum</i>	KU196084.1	100
Bd-2		<i>Enterococcus phoeniculicola</i>	GQ337034.1	99
Bd-3		<i>Enterococcus avium</i>	LC096212.1	99
Bd-4		<i>Enterococcus termitis</i>	CQ337037.1	99
Bd-5		<i>Vagococcus fluvialis</i>	JF690756.1	99
Bd-6	Streptococcaceae	<i>Lactococcus lactis</i>	KY438201.1	100
Bd-7		<i>Lactococcus garvieae</i>	KX671996.1	99
Bd-8	Bacillaceae	<i>Lysinibacillus macroides</i>	KT818804.1	99
Bd-9	Enterobacteriaceae	<i>Klebsiella pneumoniae</i>	KR822242.1	99
Bd-10		<i>Morganella morganii</i>	KU942484.2	99
Bd-11		<i>Citrobacter freundii</i>	CP018810.1	99
Bd-12	Myroidaceae	<i>Myroides odoratimimus</i>	EU373425.1	99
Bd-13	Microbacteriaceae	<i>Microbacterium</i> sp.	DQ337515.1	100

developmental time, the longest larval developmental duration was recorded by insects reared on diets supplemented with *L. lactis* (Ll) ($P < 0.001$) with an average developmental time of 9.73 days. An average of larval developmental time in CK was 8.83 days.

Pupal developmental duration In general, most of the treatments increased the average pupal developmental time though this increase was not significant (Fig. 1b). The *L. lactis* (Ll) and *Microbacterium* sp. (Mic) treatments with longest larval developmental time, had the shortest pupal developmental duration ($P = 0.307$). However *L. macrolides* (Lm) treated pupa recorded a significantly increased pupal developmental duration ($P = 0.04$).

Total developmental duration Larval diets supplemented with *E. phoeniculicola* (Ep) had a significant decrease in the total development duration of the pre-adult life stages ($P = 0.01$). Contrarily, larvae reared on *Microbacterium* sp. (Mic), *K. pneumoniae* (Kp), *V. fluvialis* (Vf) and *M. morganii* (Mm) treatments had a significantly longer developmental time ($0.001 \leq P \leq 0.05$), with the longest larval developmental duration was observed with flies on diets supplemented with *L. lactis* (Ll) ($P < 0.001$) with an average developmental time of 19.73 days (Fig. 1c).

3.3 The effect of bacteria on insect survival

Larval survival In general, bacterial enriched diets had no significant effect on larval survival (eggs surviving to pupal stage). Bacteria diets enriched with *E. phoeniculicola* (Ep) recorded the highest percentage of surviving larvae while *V. fluvialis* treatments had the lowest percentage of surviving larvae (Table 2). However *V. fluvialis* (Vf) and

E. phoeniculicola (Ep) treatments were not statistically different from the CK ($F = 1.203$, $P = 0.31$).

Pupal survival *Enterococcus* spp. treatments in addition to *L. macrolides* (Lm), *C. freundii* (Cf) and *M. odoratimimus* (Mm) increased the number of surviving pupae. However, this increase was not significantly different within treatments (Table 2). All other treatments recorded a reduced number of pupae surviving to adulthood, these reductions were statistically significant in *L. lactis* (Ll) ($P < 0.001$), *Microbacterium* sp. (Mic) ($P < 0.01$) and *K. pneumoniae* (Kp) ($P < 0.05$).

Total survival The total number of eggs surviving to adulthood was significantly different among treatments ($F = 2.06$, $P = 0.001$). None of the treatments recorded a significant increase in the number of pupal eclosion (Table 2). However, a significantly decrease number (21%) of pupal eclosion was recorded in *L. lactis* (Ll) treatment ($P < 0.05$).

Effect of bacteria on pupal weight The bacteria supplemented diets had significant effects on the pupal weight of treated flies ($F = 5.166$, $P < 0.001$). The *E. phoeniculicola* (Ep) and *E. termitis* (Et) significantly increased the pupal weight of the fly ($P = 0.003$ and $P = 0.012$, respectively) (Fig. 2). On the other hand, the lowest pupal weight was recorded from treatments supplemented with *K. pneumoniae* (Kp) ($P = 0.027$).

Fig. 1 The effects of bacterial supplemented larval diets on the developmental duration of *Bactrocera dorsalis*. **a** Larval developmental duration, **b** Pupal developmental duration, **c** Total developmental duration. The column mark on top indicates significant difference following post hoc tests at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. For treatment abbreviation were Control = CK, *E. gallinarum* = Eg, *E. phoeniculicola* = Ep, *E. avium* = Ea, *E. termitis* = Et, *L. lactis* = Ll, *L. garvieae* = Lg, *V. fluvialis* = Vf, *L. macrolides* = Lym, *K. pneumoniae* = Kp, *M. morganii* = Mm, *C. freundii* = Cf, *M. odoratimimus* = Myo and *Microbacterium* sp. = Mic

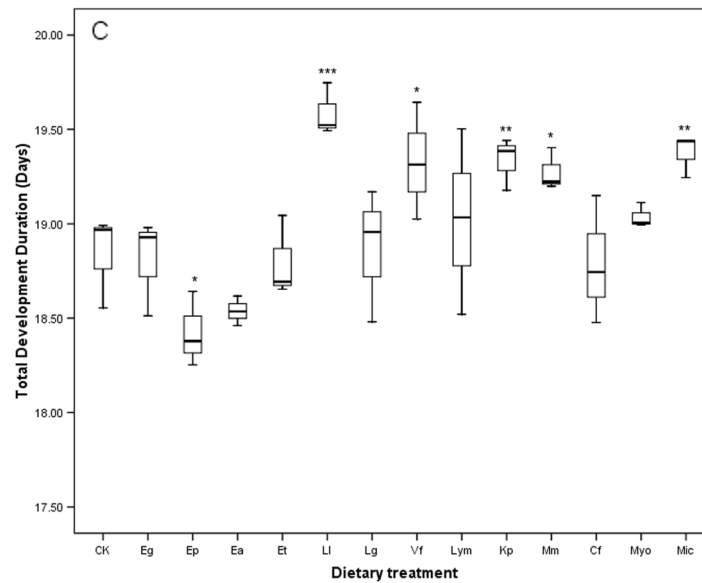
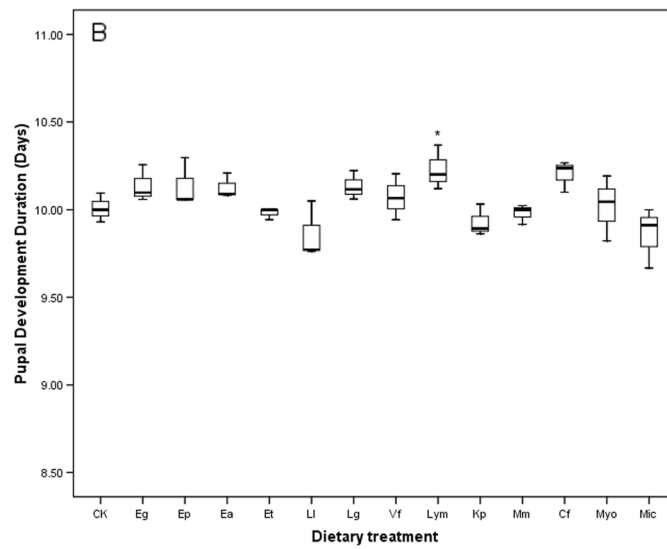
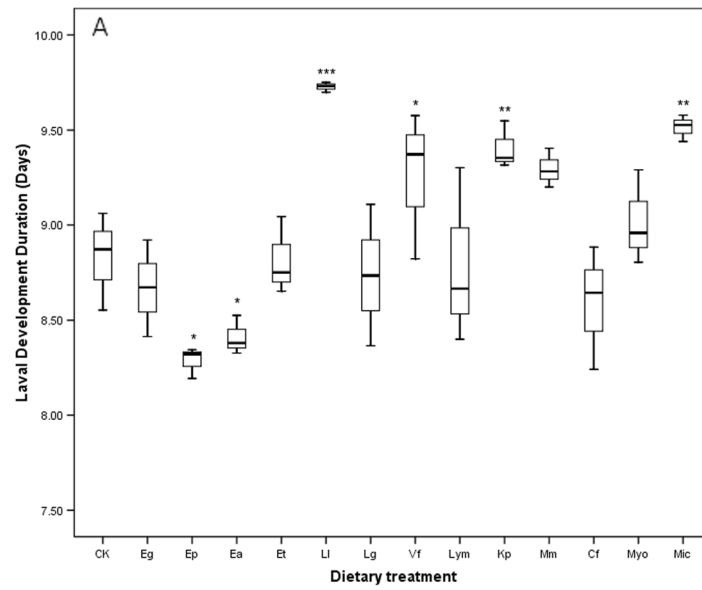


Table 2 Oriental fruit fly survival at different developmental stages

Genus	Species		Larval recovery (%)	Pupa recovery (%)	Total survival (%)
Enterococcus	Control	CK	50.3 ± 2.4	87.5 ± 1.1	44.0 ± 1.5
	<i>E. gallinarum</i>	Eg	48.3 ± 5.8	91.1 ± 2.4	44.0 ± 5.1
	<i>E. phoeniculicola</i>	Ep	57.0 ± 0.6	91.2 ± 1.8	52.0 ± 1.2
	<i>E. avium</i>	Ea	53.7 ± 2.7	94.6 ± 1.8	50.7 ± 2.2
	<i>E. termitis</i>	Et	43.3 ± 3.8	90.3 ± 6.3	39.0 ± 4.0
Lactococcus	<i>L. lactis</i>	Ll	47.7 ± 2.6	44.2 ± 1.9***	21.0 ± 0.6***
	<i>L. garvieae</i>	Lg	52.0 ± 1.7	81.8 ± 4.5	42.7 ± 3.8
Vagococcus	<i>V. fluvialis</i>	Vf	41.7 ± 8.7	75.5 ± 9.1	33.0 ± 9.9
Lysinibacillus	<i>L. macroides</i>	Lym	53.7 ± 3.8	89.9 ± 3.6	48.0 ± 1.5
Klebsiella	<i>K. pneumoniae</i>	Kp	52.0 ± 2.6	69.7 ± 10.3*	36.7 ± 7.2
Morganella	<i>M. morganii</i>	Mm	47.7 ± 1.2	76.0 ± 6.5	36.3 ± 3.8
Citrobacter	<i>C. freundii</i>	Cf	49.7 ± 4.2	93.8 ± 2.6	46.7 ± 4.4
Myroides	<i>M. odoratimimus</i>	Myo	51.3 ± 2.0	88.5 ± 3.4	45.3 ± 0.9
Microbacterium	<i>Microbacterium</i> sp.	Mic	54.0 ± 2.1	64.5 ± 4.7**	35.0 ± 3.8

The significantly different following post hoc tests at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

Association of weight, development time and survival The correlation analysis between wet weight and physiological responses of the host shows that the pupal weight had a significant negative correlation with the larval development time (Table 3). The weight increased as the developmental time reduces ($r = -0.725$, $p = 0.001$). On the other hand, there was a significant positive correlation between weight and pupal developmental duration ($r = 0.586$, $p = 0.008$) and weight

and larval survival rate ($r = 0.617$, $p = 0.005$). With an increase of pupal wet weight corresponding with an increase in the pupal development duration and larval survival rates. There was no correlation between the pupal weight and pupal survival rate ($r = 0.151$, $p = 0.538$).

4 Discussion

This study presents a culture dependent identification and assessment of the roles that gut associated symbiotic bacteria play in the development and survival of the Oriental fruit fly, *B. dorsalis*. The bacteria isolates identified in this study could be assigned to four different phyla, namely: Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria. Most of the bacteria isolated from the gut of Tephritids mainly belong to the above mentioned phyla (Wang et al. 2011; Aharon et al. 2013; Wang et al. 2014a; Augustinos et al. 2015).

From literature symbiotic bacteria has been reported to enhance survival of Tephritids (Nash and Chapman 2014) by

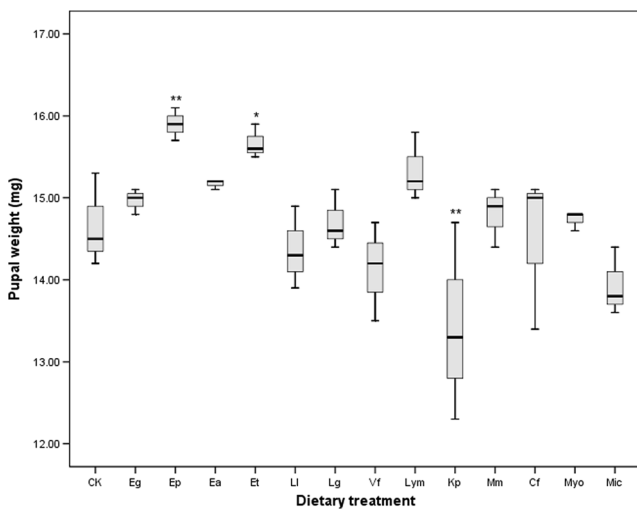


Fig. 2 Pupal weight of *Bactrocera dorsalis* reared on larval diets supplemented with different bacterium. The black line at the center of each box represents the median value. The column mark on top indicates significant differences at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. For treatment abbreviation were Control = CK, *E. gallinarum* = Eg, *E. phoeniculicola* = Ep, *E. avium* = Ea, *E. termitis* = Et, *L. lactis* = Ll, *L. garvieae* = Lg, *V. fluvialis* = Vf, *L. macroides* = Lym, *K. pneumoniae* = Kp, *M. morganii* = Mm, *C. freundii* = Cf, *M. odoratimimus* = Myo and *Microbacterium* sp. = Mic

Table 3 The correlation between wet weight, development duration and percentage survival. The top number denotes the Pearson Correlation (r) and the bottom number is the associated P -value

		Development time		Survival rate	
		Larvae	Pupae	Larvae	Pupae
Weight	Pearson Correlation (r)	-0.725**	0.586**	0.617**	0.151
	Sig. (2-tailed) (p)	0.001	0.008	0.005	0.538

**Correlation is significant at the 0.01 level (2-tailed)

providing beneficial nutrients or boosting immunity (Ben-Yosef et al. 2014; Ben-Yosef et al. 2015). The results from this study revealed that flies fed with diets containing *E. phoeniculicola* and *E. termitis* recorded an increase in pupal weight. Pupal weight gain has been shown to be affected by amino acid content in diets (Nestel et al. 2004). Our results suggest that supplementing artificial diets with *E. phoeniculicola* and *E. termitis* may have induced some physiological or behavioral changes that resulted in a corresponding increase in pupal weight. In a similar study, beetles harboring an *Enterococcus* bacterium had the ability to consume more food (Lundgren and Lehman 2010). Acquiring a higher pupal weight in mass reared fruit flies is greatly beneficial as it has been reported to enhance male competitiveness (Hamden et al. 2013).

Another relevant finding from this study is that supplementing the diet with *E. phoeniculicola* significantly reduced the larval development duration. Augustinos et al. 2015 reported a similar finding with *Ceratitidis capitata*. The faster an insect reaches a stable critical weight, the shorter the larval development time (Davidowitz and Nijhout 2004; Sentinella et al. 2013). This result suggested that *E. phoeniculicola* mediates the shortening of the larval development stage by enabling the host to gain more access to the available sources of food and efficiently assimilating the nutrients from it. A reduction in developmental time and an improvement in pupal weight may have come about by the symbiotic bacteria helping its host to extend their nutritional range (Wong et al. 2014). Either by improving digestion efficiency and/or by providing digestive enzymes or vitamins (Ben-Yosef et al. 2014) which are ultimately manifested in the development and survival of the insect hosts (Nash and Chapman 2014). Therefore, this study confirms that gut bacteria should be considered as one of the most important factors influencing the growth of their associated flies. Similarly, in *Drosophila*, gut bacteria has been reported to play a lot of roles that promotes larval growth and reduce their developmental duration (Storelli et al. 2011; Tower 2011; Strigini and Leulier 2016) in addition to facilitating in the fly's metabolic response system (Wong et al. 2014). They concluded that these associations could directly promote the insect's sensing system controlling hormonal growth signaling. However, though the consumption of bacterial supplemented larval diets significantly affected the larval developmental time, it did not have an effect on the pupal developmental time (Fig. 1). On the other hand, bacteria supplemented diets did not statistically increase the mean insect survival. This could be due to the fact that the insects were raised on a balanced diet. Difference in larval survival in *Drosophila* were only realized when larvae were raised in an unbalanced diet (Wong et al. 2014).

The insect-bacteria relationships are not always beneficial to the flies. In some cases, bacteria symbionts could delay insect development and survival (Lazzaro et al. 2006; Olcott

et al. 2010; Sarakatsanou et al. 2011). In the present study, the inoculation of *Microbacterium* sp., *K. pneumoniae*, *V. fluvialis*, *L. lactis* and *M.morganii* in larval diets significantly extended the larval developmental time (Fig. 1a). Moreover, the inoculation of bacterium *L. lactis* was the most detrimental by significantly extended the flies development duration and reduced the total host survival. The developmental duration and survival rate of flies maybe influenced by their nutrient uptake during the larval stage (Nash and Chapman 2014). From this point of view, the ingestion of *L. lactis* impairs the larva from feeding or prohibits it from making maximum utilization of the diet it consumes thereby explaining the longer larval developmental time. A previous study suggested that *L. lactis* is a pathogenic bacterium in *Drosophila* that causes fly mortality (Lazzaro et al. 2006). It has also been reported as a pathogen that cause high numbers of insect mortality through their host immune response system or disease occurrence (Linder et al. 2008; Galac and Lazzaro 2011; Aharon et al. 2013). Therefore, *L. lactis* might be pathogenic by causing injuries to the host tissues that led to the reduction of host survival.

5 Conclusion

Our study presents the effects different gut bacteria isolates have on the development of the Oriental fruit fly, *B. dorsalis*. Supplementing the larval diets with *E. phoeniculicola* could be beneficial in improving the pupal weight and reducing the larval developmental time. On the other hand, larval diets supplemented with *L. lactis* could have a pathogenic effect. Therefore, it could be concluded that the roles different bacteria play in their relationship with *B. dorsalis* is unique and varies across species. Understanding these roles is important in developing an efficient integrated pest management strategies and effectively application of SIT programs to combat these pests.

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Author's contribution CYN and PG design and supervised the experiments; KK, AAA, MA and BS carried out the research and wrote the manuscript; KK BS and ZJ analyzed the data; All the authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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