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Isolation and Characterization of Bacterial Gut Symbionts from Irradiated, Wild and Lab Reared Males of Melon Fly, *Zeugodacus cucurbitae* (Coquillett)

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ABSTRACT

The melon fly, *Bactrocera cucurbitae* is of serious concern inflicting heavy losses to the horticultural industry. It attacks cucurbits right from the primordial stages of the crop up to harvest and causing yield loss of 30 to 100%. The Sterile Insect Technique is a species-specific and environmentally non-polluting method of insect control that relies on sterilization of males using gamma rays and systemic release of sterile males in wild environment. Male pupae of *Zeugodacus cucurbitae* were exposed to gamma radiation at 50 Gy using Cobalt-60 source. Bacterial gut symbionts from sterile males, wild males of field collected population and laboratory reared males of melon fly were isolated and characterized based on morphological characteristics (*viz.,* colour, size, shape, opacity, margin, elevation and viscosity), gram staining, morphology and arrangement of bacterial cells in culture media. Ten adult flies from sterile, wild and laboratory reared males were used for isolation of gut symbionts. The most dominant phylum of bacteria found among the sterile, wild and lab reared male flies was Proteobacteria followed by the phylum Firmicutes. Different genera of bacteria isolated from sterile males were *Enterobacter, Providencia* and *Bacillus.* From wild males; *Enterobacter, Providencia, Morganella, Klebsiella* and *Bacillus* were identified. Bacterial genera obtained from lab reared males were *Enterobacter, Providencia, Klebsiella* and *Bacillus.* Among the entire bacterial genus, *Enterobacter, Providencia* and *Bacillus* were the common bacterial genera isolated from sterile, wild and lab reared male flies. Irradiation had resulted in loss of endosymbiotic bacteria in sterile males.

Keywords: Melon fly, wild males, lab reared males, sterile males, gamma radiation, bacterial gut symbionts.

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INTRODUCTION

The Melon fly, *Zeugodacus cucurbitae* (Coquillett) (Tephritidae: Diptera) is one of the world's most economically important horticultural and quarantine insect pests causing significant losses to the horticultural industry (Vargas, Pinero, & Leblanc, 2015). It is found worldwide, but India is considered to be its native habitat. Melon fly is a serious pest on cucurbits but it has also been found in beans, tomatoes and other commercial crops. It attacks 81 plant species belonging to 19 different families and destroys more than 60% of cucurbit crops in India (Kapoor, 2005). Cucurbits are subjected to damage by Melon fly right from the primordial stages of the crop up to harvest and yield loss due to fruit fly damage varies from 30 to 100%, depending on the population of fruit fly and cultivar type (Viraktamath, Mallik, Chandrashekar, Ramakrishna, & Praveen, 2003). The insect-bacterial association between hexapod and gut bacteria has complex interactions and is essential in providing natural sources of nitrogen, amino acids and vitamins which are lacking in host fruits, gut physiology and reproduction (Douglas, Minto, & Wilkinson, 2001). The microbiota of *Enterobacteriaceae* were found to influence several biological traits of Med fly, such as shortening of immature developmental stages, increasing fecundity, extending survival, increasing male mating competitiveness and increasing female mating receptivity (Deutscher, Chapman, Shuttleworth, Riegler, & Reynolds, 2019). Volatiles produced by the microbial symbionts play a crucial role in insect communication and production of pheromone. The sex pheromones trimethylpyrazine (TMP) and tetramethylpyrazine (TTMP) produced in the male rectum of *Bactrocera dorsalis* (Hendel) showed a strong attraction to wild females (Ren, Yingao, Mingxue, Yongyue, & Cheng, 2021). The bacteria within the intestine of adult flies have crucial functions in host development and reproduction and they significantly contribute to overall host fitness (Ben-Yosef, Jurkevitch, & Yuval, 2008). Endosymbiotic bacteria also known to influence host gut physiology, tissue homeostasis and environmental stress tolerance, as well as host resistance to pesticides and pathogens (Broderick, Buchon, & Lemaitre, 2014; Cheng et al, 2017).

Insect arthropods have a symbiotic relationship with microorganisms particularly bacteria as endosymbionts. Microbial symbionts play an important role in biology of many insects influencing insect nutrition, immunity, reproduction, ecology and evolution. Fruit flies have a variety of bacterial symbionts in their digestive system influencing a variety of developmental and fitness parameters. This functional contribution of symbiotic microorganisms to insect physiology could be useful in mass-rearing facilities. Bacterial strains isolated from microbiota of wild individuals can be given as supplements to mass reared insects in an attempt to replicate the natural microbiome and improve fitness and mating success (Ras, Beukeboom, Caceres, & Bourtzis, 2017). Mass rearing and irradiation of male flies may undermine the quality and mating vigour of the flies due to adverse effects of gamma radiation. Isolation and identification of beneficial bacterial endosymbionts from wild males of fruit flies will helpful in improving the fitness of irradiated and lab reared and males by providing in the form of probiotic diets (Hamden, Guerfali, Fadhl, Saidi, & Chevrier, 2013; Kyritsis, Augustinos, Caceres, & Bourtzis, 2017).

The process of radiation may undermine the quality of sterile males by affecting their mating vigour and competitiveness. Furthermore, the poor fitness and field performance of sterile males caused by irradiation may be associated with injured tissues and condition of the bacterial symbionts of their digestive system (Lauzon & Potter, 2012). The bacteria present in released males after mass rearing and irradiation may differ from their wild counterparts which impair their mating performance. Thus, restoring key symbiotic bacteria in mass-reared sterile flies prior to release is essential for improving the efficiency of Sterile Insect Technique (SIT). Providing symbiotic bacteria in pre-release adult holding diet in the form of probiotics improves the sexual performance of the released sterile males in the field. The use of semiochemicals and probiotic treatments increased the competing ability of sterile males in fruit flies (Hamden et al*,* 2020). Hence, it is necessary to know the bacterial communities in Melon fly to enhance the effectiveness of male sterile insect technique. The present work was therefore undertaken to identify and characterize the gut bacterial communities of irradiated, wild and lab reared males of *Z. cucubitae* using culture dependent approach.

MATERIALS AND METHODS

The studies on isolation and characterization of bacterial gut symbionts of *Z. cucurbitae* was conducted during 2021-22 at Post Graduate (PG) research laboratories of Department of Entomology and Plant Pathology, S.V. Agricultural College, Tirupati, India.

Pupae required for irradiation were raised by rearing larvae of Melon fly on larval liquid diet (Panduranga, Sharma, & Sharma, 2018). The male pupae of 48 hours before adult eclosion were packed carefully in plastic petri plates and exposed to gamma radiation (Cobalt60 source) at 50 Gy using gamma chamber (GC- 5000, BRIT and AERB) at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka. Wild flies were obtained from the Melon fly infested bitter gourd (*Momordica charantia* L.) fruits from field. Infested fruits were brought to the laboratory and were kept at controlled conditions (25-27°C, 65-75% RH) in 8×6″ glass jars provided with 5 cm thickness of sterilized sand for pupation. The fully-grown larvae popped out from the fruit into soil for pupation. Pupae were collected and transferred to adult rearing cages (30×30×30cm). Adults were provided with a mixture of sugar and yeast hydrolysate (3:1) as adult diet and water-soaked cotton swabs in 100 ml conical flask as source of water. Cages were cleaned and replaced with adult diet and water as and when necessary. Laboratory reared male flies were obtained by providing natural ripened pumpkin host fruits to initial Melon fly culture maintained in the laboratory for oviposition. Pumpkin fruits oviposited by melon flies were collected and transferred into plastic rearing trays (60×40×7.5cm) with 5 cm thickness of sterilized soil and the trays were covered with black cotton cloth secured by rubber band. Ripened pumpkin fruits were provided to the developing maggots on alternate days. The fully-grown larvae popped out from fruit into soil for pupation and pupae were collected and transferred to adult rearing cages (30×30×30cm). Adults were provided with a mixture of sugar and yeast

hydrolysate (3:1) in small petri plates as adult diet and water-soaked cotton swabs in 100 ml conical flask as source of water.

Ten adult male flies from sterile, wild and laboratory reared *Zeugodacus cucurbitae* were used for isolation of bacterial gut symbionts by following procedures described by Lloyd, Drew, Teakle, & Hayward (1986). Sterile, wild and laboratory reared live male flies were taken separately in glass vials and kept in a refrigerator at 5°C for 5 min to cold-anesthetize and to prevent regurgitation of gut fluid. Before dissection of flies, the flies were surface sterilized by immersing in 70% ethanol for 30 seconds and then flies were dipped in 0.25% sodium hypochlorite for 1 minute. Finally flies were washed thrice with distilled water to remove the external contamination of microorganisms. The surface-sterilized male flies were individually dissected in sterile agar-agar plates under laminar air flow using a stereomicroscope (Olympus SZ 61 with magnification of 5x). The dissected alimentary tract's midgut (Fig. 1) was carefully separated and squeezed with a sterile glass rod (Tauc, Tasdogan, & Pandur, 2014).

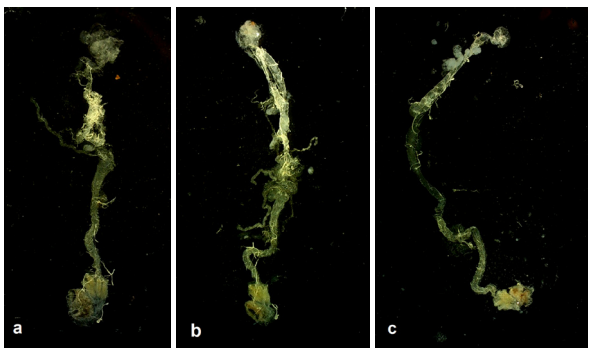

Figure 1. Dissected gut sample from a) sterile male, b) wild male, c) lab reared male of *Zeugodacus cucurbitae*

A loopful of squeezed gut fluid from sterile, wild and lab reared males were streaked separately on Nutrient Agar (NA) media in different petri dishes and were incubated for 24-48 hours in BOD incubator at 34°C. After 14-days, predominant bacterial isolates from each culture were obtained through repeated sub-culturing to obtain pure culture. The purified individual bacterial isolates were preserved in agar slants for further characterization (Fig. 2).

For morphological characterization of bacterial gut symbionts; purified bacterial isolates were screened based on colony characteristics (*viz.,* colour, size, shape, opacity, margin, elevation and viscosity), Gram staining, morphology and arrangement of cells. Obtained isolates were identified using Bergey's Manual of Determinative Bacteriology (Whitman et al, 2012). Gram staining technique was used to differentiate Gram-positive and Gram-negative bacteria. A Gram-positive bacterium stains purple, while Gram-negative bacterium stains pink or red when subjected to Gram staining. The Gram-staining technique was performed by following the method of Claus (1992). The shape and arrangement of bacterial colonies were obtained by capturing images

using Olympus CX 41 microscope equipped with a Magcam DC 5 digital camera (5.1 MP, 1/ 2.5″ CMOS sensor) at the magnification level of 100x /1.25 oil**.** The length and width of the spores of all the bacterial isolates were also measured using the above mentioned microscope. Colony characteristics *viz.,* colour, size, shape, opacity, margin, elevation and viscosity of the pure isolates colonies were observed under light microscope.

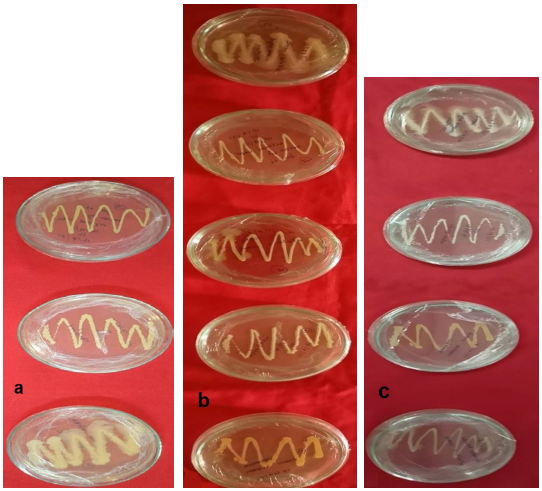

Figure 2. Sub cultures of bacterial isolates from gut samples of a) sterile, b) wild, c) lab reared males of *Zeugodacus cucurbitae*.

RESULTS

Morphological characteristics of bacterial gut symbionts isolated from sterile males of melon fly (BCS) are presented in table 1 and depicted in fig. 3. Colony and cell morphology of three different isolates were recorded.

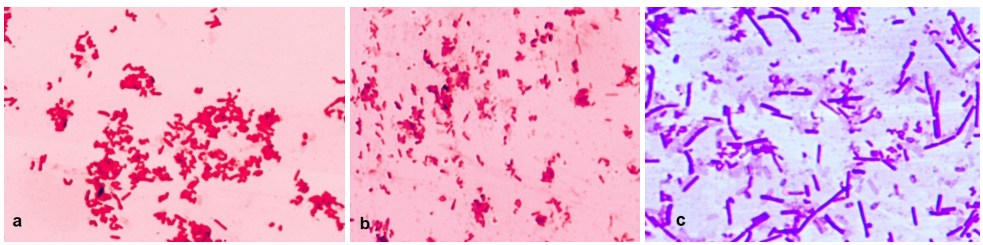

Figure 3. Morphological characterisation of bacterial gut symbionts from sterile males of *Zeugodacus cucurbitae*. a) *Enterobacter*, b) *Providencia*, c) *Bacillus*.

Colonies of BCS1 (Bacterial culture of sterile males) isolate were Gram-negative, rod shaped, large, flat, translucent, dull yellow colour with raised and convex elevation and irregular edges. BCS2 isolate were large, circular, translucent, moist, creamy white colour with convex elevation and entire margin. Isolate of BCS3 colonies were

Gram-positive, rod shapes, large, circular, opaque, dry, white colour with flat elevation and irregular margin (Table 1). Based on morphological characteristics, the isolates such as BCS1, BCS2 and BCS3 obtained from the gut of sterile male flies were identified as *Enterobacter, Providencia,* and *Bacillus,* respectively (Table 2).

Table 1. Morphological characterization of bacterial gut symbionts from sterile males of *Zeugodacus cucurbitae*

Isolates	Colony morphology							Gram staining	Cell morphology		Cell arrangement
	Size	Shape	Opacity	Texture	Elevation	Colour	Margin		Size (µm)	Shape	
BCS1	Large	Flat	Translucent	Mucoid	Raised	Dull yellow	Irregular	Negative	0.75 X 1.72	Rod	Single
BCS2	Large	Circular	Translucent	Moist	Convex	Creamy white	Entire	Negative	0.73 X 1.79	Rod	Single and pairs
BCS3	Large	Circular	Opaque	Dry	Flat	White	Irregular	Positive	1.13 X 5.7	Rod	Single

BCS1-Bacterial culture from sterile males isolate 1; BCS2-Bacterial culture from sterile males isolate 2; BCS3-Bacterial culture from sterile males isolate 3.

Table 2. Bacterial gut symbionts from sterile males of *Zeugodacus cucurbitae.*

Isolate	Identification	Family	Phylum
BCS1	Enterobacterbacter	Enterobacteriaceae	Proteobacteria
BCS2	Providencia	Enterobacteriaceae	Proteobacteria
BCS3	Bacillus	Bacillaceae	Firmicutes

BCS1-Bacterial culture from sterile males isolate 1; BCS2-Bacterial culture from sterile males isolate 2; BCS3-Bacterial culture from sterile males isolate 3.

Morphological characteristics of bacterial gut symbionts isolated from field collected population (wild male flies) of melon fly (BCW) are presented in table 3 and fig. 4. Five bacterial isolates were obtained from wild male flies were observed for colony and cell morphology. Colonies of isolate BCW1, BCW2, BCW3 and BCW4 (Bacterial culture of wild males) are Gram-negative, rod shaped bacteria cells that were large, flat, translucent. Colour of the colonies of BCW1, BCW2, BCW3 and BCW4 are dull yellow, creamy white, opaque and white in colour, respectively with raised and convex elevation. Colonies of BCW5 were Gram-positive, rod shaped single cells, large, circular, dry, white colour with flat elevation and irregular margin (Table 3). Based on the characteristics of the isolates; BCW1, BCW2, BCW3, BCW4, and BCW5 obtained from the gut of *Z. cucurbitae* wild males were identified as *Enterobacter, Providencia, Morganella, Klebsiella* and *Bacillus,* respectively (Table 4).

Table 3. Morphological characterization of bacterial gut isolates from wild males of *Zeugodacus cucurbitae.*

Isolates	Colony morphology							Gram staining	Cell morphology		Cell arrangement
	Size	Shape	Opacity	Texture	Elevation	Colour	Margin		Size (µm)	Shape	
BCW1	Large	Flat	Translucent	Mucoid	Raised	Dull yellow	Irregular	Negative	0.73 X 1.73	Rod	Single
BCW2	Large	Circular	Translucent	Moist	Convex	Creamy white	Entire	Negative	0.71 X 1.78	Rod	Single and pairs
BCW3	Small	Circular	Opaque	Mucoid	Convex	White	Entire	Negative	0.66 X 1.15	Rod	Single
BCW4	Large	Circular	Opaque	Mucoid	Convex	Creamy white	Entire	Negative	0.75 X 1.81	Rod	Single, pairs and short chains
BCW5	Large	Circular	Opaque	Dry	Flat	White	Irregular	Positive	1.12 X 5.5	Rod	Single

BCW1-Bacterial culture from wild males isolate 1; BCW2-Bacterial culture from wild males isolate 2; BCW3-Bacterial culture from wild males isolate 3; BCW4-Bacterial culture from wild males isolate 4; BCW5-Bacterial culture from wild males isolate 5.

Table 4. Bacterial gut symbionts from wild males of *Zeugodacus cucurbitae*.

Isolate	Identification	Family	Phylum
BCW1	Enterobacterbacter	Enterobacteriaceae	Proteobacteria
BCW2	Providencia	Enterobacteriaceae	Proteobacteria
BCW3	Morganella	Enterobacteriaceae	Proteobacteria
BCW4	Klebsiella	Enterobacteriaceae	Proteobacteria
BCW5	Bacillus	Bacillaceae	Firmicutes

BCW1-Bacterial culture from wild males isolate 1; BCW2-Bacterial culture from wild males isolate 2; BCW3-Bacterial culture from wild males isolate 3; BCW4-Bacterial culture from wild males isolate 4; BCW5-Bacterial culture from wild males isolate 5

.Morphological characteristics of bacterial gut symbionts isolated from lab reared males of *Z. cucurbitae* (BCL) are presented in table 5 and fig. 5. Colonies of BCL1, BCL2, and BCL4 (Bacterial culture of lab reared males) isolates were Gram-negative, rod shaped, single occurred cells. Colonies are larger in size, circular - flat shape, translucent with mucoid viscosity. The colour of BCL1, BCL2, and BCL4 are dull yellow (irregular margin), creamy white colour with convex elevation, respectively. Whereas BCL3 colonies were Gram-positive, rod shaped, large, circular, opaque, white colour with and irregular margin (Table 5). Based on the morphological characteristics of BCL1, BCL2, BCL3 and BCL4 isolates from the lab reared sterile males were identified as *Enterobacter, Providencia, Bacillus* and *Klebsiella,* respectively (Table 6). The loss of endosymbionts in sterile males was mainly due to the gamma radiation.

Table 5. Morphological characterization of bacterial isolates from lab reared males of *Zeugodacus cucurbitae*

Isolates	Colony morphology							Gram staining	Cell morphology		Cell arrangement
	Size	Shape	Opacity	Texture	Elevation	Colour	Margin		Size (µm)	Shape	
BCL1	Large	Flat	Translucent	Mucoid	Raised	Dull yellow	Irregular	Negative	0.74 X 1.75	Rod	Single
BCL2	Large	Circular	Translucent	Moist	Convex	Creamy white	Entire	Negative	0.73 X 1.78	Rod	Single and pairs
BCL3	Large	Circular	Opaque	Dry	Flat	White	Irregular	Positive	1.13 X 5.6	Rod	Single
BCL4	Large	Circular	Opaque	Mucoid	Convex	Creamy white	Entire	Negative	0.74 X 1.83	Rod	Single, pairs and short chains

BCL1-Bacterial culture from lab reared males isolate 1; BCL2-Bacterial culture from lab reared males isolate 2; BCL3-Bacterial culture from lab reared males isolate 3; BCL4-Bacterial culture from lab reared males isolate 4.

Table 6. Identification of bacterial gut symbionts from lab reared males of *Zeugodacus cucurbitae*.

Isolate	Identification	Family	Phylum
BCL1	Enterobacterbacter	Enterobacteriaceae	Proteobacteria
BCL2	Providencia	Enterobacteriaceae	Proteobacteria
BCL3	Bacillus	Bacillaceae	Firmicutes
BCL4	Klebsiella	Enterobacteriaceae	Proteobacteria

BCL1-Bacterial culture from lab reared males isolate 1; BCL2-Bacterial culture from lab reared males isolate 2; BCL3-Bacterial culture from lab reared males isolate 3; BCL4-Bacterial culture from lab reared males isolate 4

CONCLUSIONS AND DISCUSSIONS

In the present study, wild males of melon flies guts harboured wide range of Enterobacteriaceae members *viz., Enterobacter, Providencia, Citrobacter, Morganella, Klebsiella.* Similarly, lab reared male flies also had diverse members of Enterobacteriaceae *viz., Morganella, Enterobacter, Providencia* and *Klebsiella.* Gut bacterial symbionts belonging to the family Enterobacteriaceae in the sterile males

were *Enterobacter* and *Providencia*. *Bacillus* belonging to the family bacillaceae and phylum Firmicutes was present in the wild, lab reared and sterile males of melon flies.

The present findings are corroborated with findings of Hadapad, Prabhakar, Chandekar, Tripathi, & Hire (2016) investigated the composition and diversity of microbial community in the midgut of the wild population of Melon fly from India. The dominant species inhabiting the Melon flies midgut were from the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, *Bacillus* and *Providencia*.

Similarly, Yong, Song, Chua, & Lim (2017) observed high abundance of Proteobacteria in Carambola fruit fly, *Bactrocera carambolae.* Chandler, Lang, Bhatnagar, Eisen, & Kopp (2011) also found that *D. melanogaster* guts were rich in Enterobacteriaceae. The present findings were in accordance with Thaochan, Drew, Hughes, Vijaysegaran, & Chinajariyawong (2010) who isolated bacteria from the midgut of *Bactrocera cacuminata* (Hering) and *Bactrocera tryoni* (Forgatt) collected from the field. They found that *Citrobacter freundii, Enterobacter cloacae,* and *Klebsiella oxytoca* are predominant species in both the fruit flies. Molecular and culture-based techniques by Yuval, Ben-Ami, Behar, Ben-Yosef, & Jurkevitch (2013) also showed that members of the Enterobacteriaceae *viz., Klebsiella sp., Enterobacter sp., Pectobacterium sp., Citrobacter freundii* and *Providencia stuartii* form the dominant populations in the gut of *Ceratitis capitata* (Wiedemann). Wang, Yao, Zheng, & Zhang (2014) identified *Bacillus cereus, Enterococcus faecalis, Enterobacter cloacae* and *Citrobacter freundii* from *B. dorsalis* populations.

Present finding showed that major bacterial isolates belongs to the phylum Proteobacteria and family enterobacteriaceae which is found in accordance with Yong et al. (2017) who also observed a high abundance of Proteobacteria phylum and *Enterobacteriaceae* family in *B. carambolae.* Andongma, Wan, Dong, Li, & Desneux (2015) used 454 pyrosequencing to identify the bacteria associated with different developmental stages of *B. dorsalis* and reported that Proteobacteria dominated in immature stages while Firmicutes dominated in adult stages. Predominant bacterial culture obtained from lab reared male flies belongs to the phylum Proteobacteria and Firmicutes and these findings are on par with Cox & Gilmore (2007) who found that laboratory reared *D. Melanogaster* contained higher proportions of *Enterobacter* (Garmmaproteobacteria) or *Enterococcus* (Firmicutes). Khan, Mahin, Pramanik, & Akter (2014) also performed different biochemical, Gram reaction and motility tests to identify the mid-gut bacterial community of laboratory reared pumpkin fly, *Bactrocera tau* (Walker) and identified eight genera under the family *Enterobacteriaceae.* Pramanik, Mahin, Khan, & Miah (2014) found thirteen bacterial species from *B. dorsalis* belonging to eleven genera. They were *Listeria, Citrobacter, Moraxella, Proteus, Streptobacillus, Enterobacter, Serratia, Vibrio, Aeromonas, Klebsiella* and *Moragnella.* Augustinos et al. (2015) also found three bacterial species *viz., Providencia sp., Enterobacter sp.,* and *Acinetobacter sp*. from medfly Vienna 8 strain using culture-dependent approach.

Wild flies had diverse gut bacterial symbionts when compared to lab reared and

sterile male flies, which was concurrent with findings reported by Wang, Jin, & Zhang (2011) that field collected flies had more diverse gut flora than laboratory reared male flies of *Bactrocera dorsalis*, which was due to more opportunities to feed on natural diets. Similar findings were reported by Estes et al. (2011) that lower bacterial diversity in laboratory reared flies of the olive fruit fly, *Bactrocera oleae* (Rossi), was most likely due to laboratory conditions and artificial diets containing antibiotics and antimicrobials. Whereas the gut bacterial symbionts obtained from sterile males (irradiated at 50 Gy) were few compared to wild and lab reared males which were due to effect of gamma radiation. These results were concurrent with findings of Yuval et al. (2013) who observed that the structure of *B. dorsalis* symbiome was significantly altered at radiation dose of 50 Gy.

The result from the present studies revealed that, diverse bacterial population was found in the gut of sterile, wild and lab reared males of Melon fly, *Z. cucurbitae.* The most dominant phylum found among all the three groups of melon flies was Proteobacteria. Next to Proteobacteria, phylum Firmicutes was observed in all the flies examined. In wild males, bacteria belongs to the phylum Proteobacteria was abundant. Dominant family among all the male flies was Enterobacteriaceae and only one member belongs to Bacillaceae. *Enterobacter*, *Providencia* and *Bacillus* were common bacterial genus isolated from wild, lab reared and sterile male flies. Genus *Morganella* was present only in the gut of wild melon flies and the genus *Klebsiella* was found in both wild and lab reared flies but absent in sterile males. These results were inclined with findings of Hadapad, Shettigar, & Hire (2019) who found that guts of wild and mass-reared *Z. cucurbitae* and *B. dorsalis* had diverse bacterial composition in varying degrees of abundance. The phylum Proteobacteria was more prevalent in wild *Z. cucurbitae* and *B. dorsalis* when compared to mass-reared colonies. The dominant family in the guts of both wild and mass-reared was Enterobacteriaceae. Changes in the bacterial symbiome of irradiated melon flies included a significant decrease in the number of sequences associated with *Citrobacter*, *Raoultella* and Enterobacteriaceae members (Asikamis et al, 2019). However, the molecular characterization of bacterial gut symbionts has to be done for their confirmation. From the study, it is concluded that the process of irradiation and use of an optimum radiation dose for sterilization of males is very crucial to induce desirable level of reproductive sterility in males without causing much damage to the harboured and acquired gut symbionts in adult males, as the endosymbionts play significant role in production of pheromones and longevity of male flies. Further research may be conducted on the supplements of beneficial gut bacterial isolates to enhance different quality parameters (mating compatibility, longevity, etc.) of sterile males of *Z. cucurbitae* in support of SIT application.

DECLARATION

The authors declare that they have no conflict of interest.

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