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CULTIVABLE GUT MICROBIAL DIVERSITY OF IRRADIATED *SPODOPTERA LITURA* (F.)

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ABSTRACT

The present study was aimed to ascertain the effect of male parent irradiation (in context of radio-genetic 'Inherited sterility technique' (IS), for *Spodoptera litura* suppression) on the cultivable gut bacteria of its F1 progeny of irradiated males (100Gy, 130Gy) in relation to control using culture dependent method. The F1 progeny were subjected to isolation of culturable microbes and 16S rDNA sequence-based identification. The Scanning electron micrographs of the F1 larval guts showed the presence of biofilms associated with bacteria, as a manifestation of stress response unlike in control. The bacteria in the control were found to belong to the genera *Bacillus, Pseudomonas,* and *Enterococci,* whereas in the irradiated F1 progeny *Bacillus* and *Pseudomonas* were predominantly present. Biochemical characterization and antibiotic tests of the bacteria showed a differential pattern in F1 progeny than in control. The bacterial abundance was increased due to irradiation stress, whereas the generic richness was apparently more in control than in irradiated regimens.

Key words: Culture dependent bacteria, 16SrRNA gene sequencing, *Spodoptera litura,* insect irradiation, microbiota, phylogenetic analysis, biochemical characterization, antibiotic tests

Microbes have an important role in the overall growth and development of insects. A panoply of microbial population inhabits the insect gut which can be either existing mutually with the host or in an obligatory relationship (Gupta and Nair, 2020). Endosymbionts have been found to impact the insect's fitness along with playing major role in the supply of essential nutrients, production of pheromone, and aids in digestion and reproduction (Singh et al., 2021). Aphids and termites which feed on a specialized niche are particularly important because of the biochemical transformation that takes place in the enzymes of the microbes (Prasad et al., 2018). The larvae of lepidopterans are mostly herbivores and the food bolus are not sterile (Dillon et al., 2004). The native gut bacteria of the insects detoxify the harmful secondary metabolites along with protecting the host from colonization of the pathogens (Dillon et al., 2010) and maintains the homeostasis of plant defense elicitors in the lepidopteran larvae. *Spodoptera litura* (F.) also known as tobacco cut worm, is an economically serious agricultural pest that feeds on most of the plant species and is polyphagous in nature (Chunxian et al., 2004). It belongs to the Noctuidae family and has a worldwide distribution. The larvae of this pest moth are gregarious feeders during the early instar and feed by scraping the leaves. Later instars can completely devour the fruits and leaves leading to a complete loss in the crop growth (Kaur and Chandi, 2021).

Spodoptera litura is a serious pest in India and it has developed resistance against a variety of insecticides used to control it (Tong, 2013). Hence, an environment friendly radio-genetic technique, Inherited Sterility Technique (IS), as a modified 'Sterile Insect Technique' (SIT) has been proposed to control various lepidopteran pests including *S. litura* (Seth and Sharma, 2001; Seth et al 2016a, b, Marec and Vreysen, 2019). A radiation dose range of 100-130Gy was proposed as partially sterilizing dose to be used in IS technique against *S. litura*, and the moths must be biologically viable to compete with wild population and transfuse the inherited sterility to tackle this pest. In this radio-genetic technique (IS), the male moths are sterilized partially by radiation but they are supposed to be behaviourally viable, so that the inherited sterility can be employed in pest control. The reproductive viability may depend on inherent characteristics, viz., metabolism, body size, age, weight, and nutrients. Insect gut flora may govern mating behaviour along with associated reproductive activities and also aid in nutritional physiology (Saour, 2014). There are no reports related to the effect of irradiation on the structure of the Lepidopteran gut bacteriome. It is documented that the gut associated

bacterial species have a correlation with the overall ecological fitness and biological quality of their host, i.e., the insects (Cai et al., 2018). Hence, there is a need to explore the influence of irradiation on insect gut diversity which might be correlated with pest's reproductive viability. In view of this, the current study in its initial phase was aimed to ascertain the cultivable bacterial community structure and composition within the gut of the *S.litura* larvae and determine the effect of male parent irradiation on the cultivable gut microbial diversity harbouring the F1 progeny pest larva (derived treated male parents), so that the irradiation impact on gut microbial diversity of F1 progeny could be correlated with its development and reproductive competence vis-à-vis control (untreated insect larvae).

MATERIALS AND METHODS

Insect culture originated from agricultural fields around Delhi was maintained in the laboratory (27± 1°C, 75± 5% RH, 12:12 hr L:D with lights on at 06.00 hr and off at 18.00 hr) on castor leaves (Seth and Sharma, 2001). Irradiation was carried out at the Institute of Nuclear Medicine and Allied Sciences (INMAS) of the Ministry of Defense in Delhi in a Co^{60} source Gamma chamber. The radiation dose rate of the source ranged from 0.693 to 0.488KGy/h. 0- 1 day old adult male moths were irradiated at 100 or 130Gy, as proposed by Seth and Sharma (2001). The irradiated male adults (100Gy♂ and 130Gy♂) were crossed with normal females (0Gy ♀) and sixth instar larvae (L6) of the F1 progeny derived from both the 100Gy♂ and 130Gy♂ male parents were selected along with F1-L6 from normal males (0Gy♂) as control.

Culturable bacteria were isolated from the larval gut. Fifteen sixth instar larvae (L6) were collected and starved for 24 hr. 70% ethanol was used for the washing of the larvae (four times) followed by 5% sodium hypochlorite (v/v) for surface sterilization. The excess of sodium hypochlorite was then removed using the autoclaved distilled water. The larvae were dissected in 1 ml of phosphate buffer saline (pH 7.0) by removing the whole gut and then it was pooled. All the steps were carried out in a Laminar flow cabinet, following aseptic conditions (Thakur et al., 2015). The bacterial 'colony forming unit' (CFU) titre was studied in the irradiated F1 larvae (100Gy F1L6 and 130Gy F1L6) as well as in control (0Gy F1L6) gut. The homogenate was serially diluted using the phosphate buffer saline.100 µl of the dilution was spread plated on Luria Bertani agar plates (10g tryptone; 5g peptone; 5g NaCl; 15g NaCl in 1 l of MQ water). The plates were incubated in a BOD incubator at 30± 2°C and the bacterial CFUs were enumerated in the F1 progeny of irradiated male parent with respect to control by plating serial dilution on LB agar. The statistical analysis was performed using Graph Pad Prism software (version 8.0). One way ANOVA was used followed by Tukey post-hoc test for multiple comparisons between the bacterial titres (CFU/ ml) under different regimen. The colonies were differentiated based on their size, colour and morphology, and a single representative isolate of each morphotype was transferred to a new plate. After five repeated streaking and re-streaking, the purified isolates were preserved in LB agar in 70% glycerol stocks at -20°C for further study.

The gut bacteria of *S.litura* larvae was studied under scanning electron microscopy. After dissection, the entire gut was fixed by dipping in 2.5% glutaraldehyde overnight at 4°C (Wipfler et al., 2015). The next day, the samples were centrifuged for 10 min at 9000 rpm in 0.1M phosphate buffer (pH 7.0) followed by dehydration in increasing concentration of ethanol series. The imaging was done at the University of Delhi, USIC. The biochemical test was performed using KB003 Hi-media kit. With 80µl of the overnight grown bacterial cultures, followed by incubation for 24 hr at 30°C (Gandotra et al., 2018; Bhat et al., 2021). When the bacterial cultures were inoculated in the test kit, the substrate present in the kit exhibits a colour change that could be interpreted either visually or by the addition of a reagent. The isolates derived from the F1 progeny of control and irradiated parent were screened for protease and lipase production enzyme. A skim milk agar plate was used for the protease enzyme assay, wherein the bacteria were point inoculated onto the skim milk agar plates and incubated under normal conditions. The bacteria capable of producing caseinates or peptidases would degrade the protein present in the milk powder. A clearing zone was obtained indicating casein digestion (Azis et al., 2019). The tween 80/20 agar plate was used for assessing the lipase activity in the isolated bacteria. The bacteria were point inoculated onto the plates, and incubated under normal conditions. The deposition of calcium crystals was noted for 3-4 days. The isolates positive for lipolytic activity produced a prominent halo of degradation around them indicating deposition of calcium salt crystals of lauric acid (Azis et al., 2019). For catalase enzyme activity, hydrogen peroxide (H_2O_2) was used. A glass slide was used to perform this test. A small amount of bacterial colony was taken and place on the glass slide and one drop of 3% H_2O_2 was placed

onto the isolates. The positive test accounted for the formation of the bubbles which was due to the oxygen production. No bubble formation was accounted for negative reaction (Reiner, 2010).

The susceptibility of bacteria for different antibiotics were checked using Kirby Bauer test. In this qualitative test, Hi-media icosahedral antibiotic discs impregnated with known concentration of antibiotics were used. The disc was kept on the agar plate, already inoculated by the test bacteria. This disc was incubated and during that period, the antibiotics could diffuse from that icosahedral disc to the agar plate. Based on the solubility of the antibiotic and its molecular size, a concentration gradient was formed on the agar. If the organism was resistant then there would be growth around the disc and if the organism was susceptible then no growth was observed. Zone of inhibition was the area of no growth around the antibiotic disc. Using the scale, the zone of inhibition was measured (Khan et al., 2019).

DNA isolation from culturable bacteria was done using the GTE method (Glucose Tris EDTA method). Polymerase chain reaction (PCR) for the genomic DNA obtained from each bacterial isolate was set up to amplify the variable regions of the 16s rRNA genes. A forward 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse 1492R (5'GGT TAC CTT GTT ACG ACT T 3') 16S rRNA primers were used. The reaction mixture of 25µl was prepared to have the concentration of 1µM for forward and reverse primer each, 25 ng DNA template, 200 µM dNTP (each), 1.5 U Taq polymerase, Taq buffer (1X), and DNase free water for making up the volume. PCR conditions followed were as initial denaturation for 5 min 94°C, followed by 30 cycles of denaturation for a min at 94°C, annealing at 55°C for one min, extension at 72°C for 2 min, and extension at 72°C for 7 min. The amplification was observed on 0.8% agarose gel with EtBr (0.5 µg ml^{-1}) in 0.5 X TBE buffer at 80 volts. PCR amplicons were identified using a 100bp DNA ladder (500µg ml^{-1}) used as a molecular weight standard marker. The gel was visualized on a UV trans-illuminator using Gel Doc (Clarridge, 2004). The amplified products were subjected to Sanger sequencing. The raw data were obtained as forward and reverse sequences. The sequences were trimmed and a consensus sequence was made using BioEdit. The obtained sequences were subjected to BLAST (Basic Local Alignment Search Tool) in NCBI/BLAST homepage (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest bacterial neighbours. The closely related bacterial species were retrieved from GenBank. MUSCLE (Multiple sequence alignment) tool was used for multiple sequence alignment in MEGA X. A neighbour-joining tree using the Maximum Likelihood method was drawn with 1000 bootstrap replicates using MEGAX software (Tamura et al., 2004; Tamura et al., 2021). One-way ANOVA followed by Tukey's multiple comparison test was performed to assess the significance among the CFU/ml in different regimens (100GyF1L6, 130GyF1L6, control). The experiment was replicated three times and a cohort of 15 larvae constituted one replicate for obtaining gut bacteria.

RESULTS AND DISCUSSION

Cultivable bacterial diversity (CFU/ml) in the F1 progeny of irradiated male parents at 100Gy and 130Gy in comparison to control when analysed revealed that the bacterial abundance i.e, the number of bacteria that could be cultured in the laboratory (bacterial count, i.e., CFU/ml), were more in the irradiated regimens (100Gy F1L6, 130Gy F1 L6) as compared to the control insect ($p < 0.05$), which indicated that in the irradiated F1 L6 gut bacterial CFU titres got increased with an increased radiation stress, the 130Gy parental irradiation had more impact than 100Gy irradiation (Fig. 1). Scanning electron micrographs in the F1 progeny of irradiated male parents at 100Gy and 130Gy in comparison to control showed that the larval guts of 100Gy F1L6

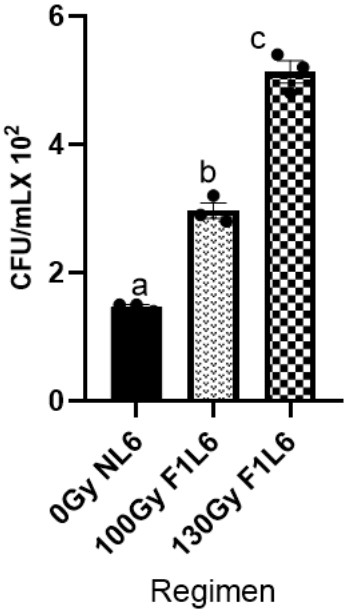

Fig. 1. Colony forming unit of bacteria (CFU/ml) in the F1 progeny of irradiated male parents at 100Gy and 130Gy in comparison to control. Bars representing Means ±SE followed by different alphabets statistically significant at $p< 0.05$ (One-way ANOVA followed by Tukey's multiple comparison test)

and 130Gy F1L6 showed the presence of a biofilm associated with bacteria in both the irradiated samples. Biofilm is generally observed in the stress conditions. The cocci shaped bacteria were distinctly visible in the control sample without any stress induced biofilm. The irradiation stress was apparent in the form of biofilm in all the irradiated regimens (Fig. 2). These biofilms are supposed to be the communities of aggregated microbial cells embedded in a self-produced matrix of extracellular polymeric substances (EPS) in response to stress (Yin et al., 2019).

Effect of irradiation on the biochemical characterization and antibiotic susceptibility in the F1 progeny of irradiated male parents and control when analysed it was observed that in the control larval gut, 36% of the biochemical enzymes were exhibited by all the four bacterial isolates, 32% of the enzymes were exhibited by ≥50% isolates, and 29% of the enzymes were not exhibited by any bacterial isolate. In the bacteria derived from the irradiated larval gut (100Gy and 130Gy), 18% of the biochemical enzymes were exhibited by all the bacterial isolates, 43% of the enzymes were exhibited by ≥50% isolates, and 40% of the enzymes were not exhibited by any bacterial isolate. Differential biochemical profiles were shown by the bacteria derived from the F1 progeny of the control and irradiated (100Gy and 130Gy) larval gut. The microbiota inhabiting the F1 progeny of the irradiated (F1) larval gut might be displaying differential metabolic activities in response to control. This might be attributed to the differential profile of biochemical enzymes exhibited by the bacterial isolates in irradiated regimens (F1 larvae) vis-à-vis control.

Bacterial inhabitants in the insect gut are of valuable significance as they increase their host survival through nutrients supplementation, by complementing the digestive enzymes or via the degradation of plant metabolites that are otherwise difficult to degrade (Gandotra et al., 2018). Insects lack a complete enzyme system and gut bacteria provide different enzymes responsible for the assimilation and digestion of nutrients (Jing et al., 2020). In this present study, the biochemical assays indicated that many of these isolates might be helping in the digestion and assimilation of nutrients of insects, Also, some bacteria might be helpful in the utilization of certain sugars. All the

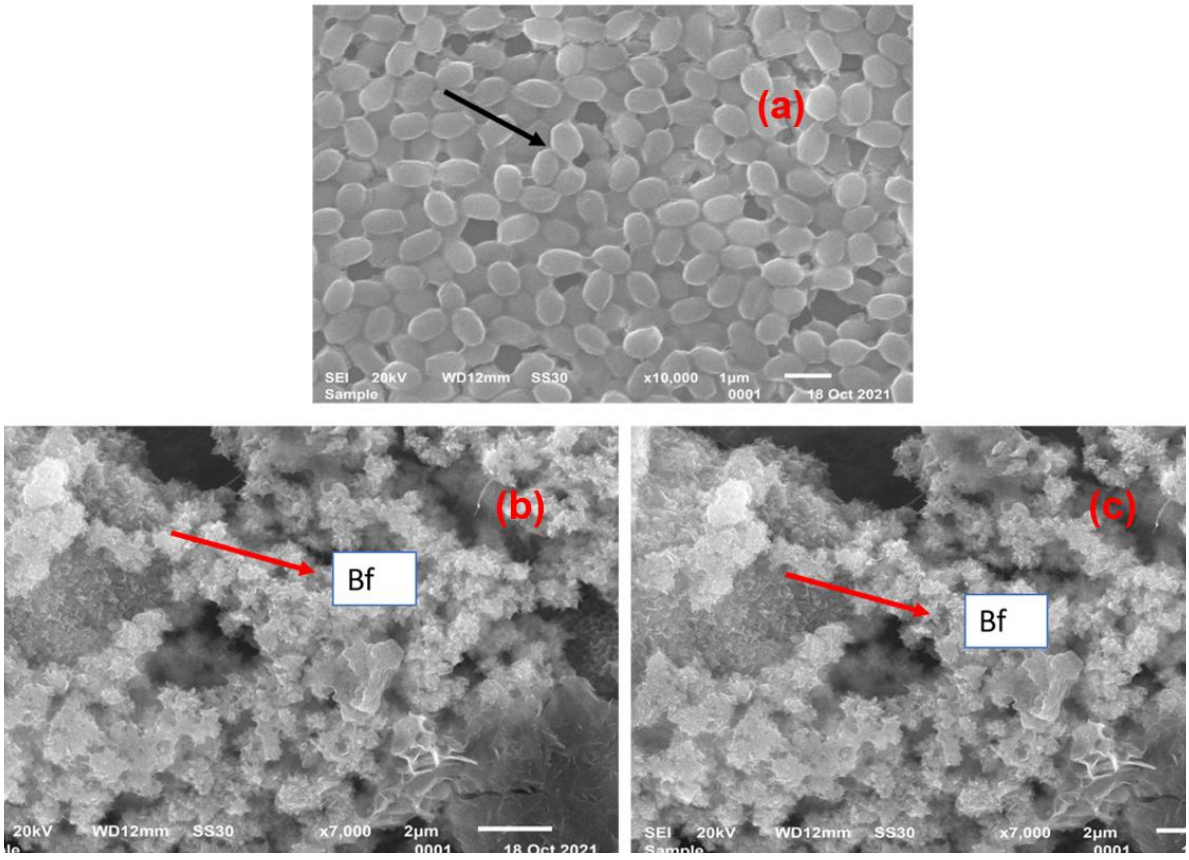

Fig. 2. Scanning electron micrographs of whole gut region of F1 progeny of irradiated male parent *S. litura,* in relation to control (a) 0Gy NL6 (Scale- 1 µm), (b) 100Gy F1L6 (Scale- 2 µm), (c) 130Gy F1L6 (Scale- 2 µm)

bacteria (isolated from irradiated regimens and control) in the present study were catalase-positive and oxidase-negative, therefore they were presumably facultative anaerobic organisms (Table 1).

Antibiotic test showed the susceptibility of bacteria derived from the F1 progeny of irradiated male parent, *S. litura,* in comparison to control. The bacteria derived from the F1 progeny of irradiated male parent, *S. litura* at 0Gy (i.e. control) showed the resistance profiles in descending order as C1> C3=C4>C2. That indicated maximum susceptibility of the C2 isolate for the antibiotics, followed by C3, C4 and C1. The bacteria derived from the F1 progeny of irradiated male parent, *S. litura* at 100Gy showed the resistance profiles as Y3>Y2>Y1. That indicated maximum susceptibility of Y1 isolate followed by Y2 and Y3. The bacteria derived from the F1 progeny of irradiated male parent, *S. litura* at 130Gy showed the resistance profiles as R2>R3>R1. The maximum susceptibility was shown by R1 isolate followed by R3 and R2. The microbiota derived from both the control and irradiated larval

Table 1. Biochemical tests of bacteria derived from F1 progeny of irradiated male parent, *S. litura* in comparison to control, to ascertain the presence of biochemical enzymes in different isolates using KB003 Hi-Media kit. (a) 0Gy NL6 (control), (b) 100Gy F1L6, (c) 130Gy F1L6

Regimen	Bacterial isolates from the Control 0Gy NL6				Bacterial isolates from the control 100Gy F1L6			Bacterial isolates from the Control 130Gy F1L6		
Biochemical test	C1	C2	C3	C4	Y1	Y2	Y3	R1	R2	R3
Oxidase production	-	-	-	-	-	-	-	-	-	-
ONPG production	-	-	-	-	-	-	-	-	-	-
Lysine utilization	-	-	-	-	-	-	-	-	-	-
Ornithine utilization	-	-	-	-	-	-	-	-	-	-
Urease production	+	-	+	-	-	+	-	-	-	+
Phenylalanine deamination	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-
Citrate utilization	+	-	-	+	-	-	-	-	-	-
Voges Proskaueuer's test	+	-	-	-	-	-	-	-	-	-
Methyl Red production	-	+	+	-	+	+	-	+	-	+
Indole production	-	-	-	-	-	-	-	-	-	-
Malonate utilization	+	-	-	+	-	-	-	-	-	-
Esculin hydrolysis	+	+	+	+	+	+	-	+	-	+
Arabinose utilization	+	+	-	+	+	+	-	+	-	+
Xylose utilization	+	+	+	+	+	+	-	+	-	+
Adonitol utilization	-	-	-	-	-	-	-	-	-	-
Rhamnose utilization	-	+	-	+	+	-	-	+	-	-
Cellobiose utilization	+	+	+	+	+	+	-	+	-	+
Mellobiose utilization	+	+	+	+	+	+	-	+	-	+
Sachharose utilization	+	+	+	+	+	+	-	+	-	+
Raffinose utilization	+	+	+	+	+	+	-	+	-	+
Trehalose utilization	+	+	-	+	+	+	+	+	+	+
Glucose utilization	+	+	+	+	+	+	+	+	+	+
Lactose utilization	+	+	-	+	+	+	-	+	-	+
Catalase test*	+	+	+	+	+	+	+	+	+	+
Protease test*	+	+	+	+	+	+	+	+	+	+
Lipase test*	+	-	+	+	+	-	+	+	-	+

*shows the tests performed using qualitative assays

gut showed different resistance profiles against the antibiotics. The microbiota in the control larval gut were more resistant to antibiotics as compared to microbiota derived from the F1 progeny of the irradiated male (100Gy and 130Gy). The bacteria derived from the irradiated larval gut showed more susceptibility towards antibiotics as they were not able to grow in the presence of antibiotics presumably because of the additional stress i.e., irradiation. In view of an overall sensitivity response of gut bacteria of normal and irradiated insects towards various antibiotics, it could be surmised that high number of isolates were susceptible to norfloxacin, gentamicin, ciprofloxacin, cefoperazonentiobiotic, and chloramphenicol. These antibiotics showed profound effects on the gut microbiota composition as high number of isolates were found to be sensitive to these drugs (Table 2).

The 16S rRNA gene amplification was carried out and approximately 1.2-1.5 kb 16S rRNA band was observed in 0.8% agarose gel. The bands were processed for Sanger sequencing. The sequenced reads were subjected to NCBI BLAST and EZ Taxon to check for the phylo-genetically similar isolates. Both the databases, viz., NCBI BLAST and EZ Taxon, are curated and were used. The phylogenetically related strains were selected and subjected to multiple sequence alignment using MUSCLE (alignment tool) in MEGA X (Software used for multiple sequence alignment and phylogenetic tree construction). A neighbor-joining tree was constructed using the Maximum Likelihood method with 1000 bootstraps (Fig. 3). The identification of the bacterial isolates were based on the 16S rRNA gene sequencing. 16S rRNA sequencing could only identify upto the genus level. Figure 3 shows the phylogentic

Table 2. Antibiotic tests of bacteria derived from F1 progeny of irradiated male parent, *S. litura* in comparison to control to ascertain the relative susceptibility or resistance of bacterial isolates towards various antibiotics. (a) 0Gy NL6 (control), (b) 100Gy F1L6, (c) 130Gy F1L6

Antibiotic tests			F1 progeny of control 0Gy NL6				F1 progeny derived from 100Gy			F1 progeny derived from 130Gy		
Antibiotics	Symbol	Conc (mcg)	C1	C2	C3	C4	Y1	Y2	Y3	R1	R2	R3
Norfloxacin	NX	10	S	S	S	S	S	S	S	S	S	S
Gentamicin	GEN	10	S	S	S	S	S	S	S	S	S	S
Chloramphenicol	C	30	S	S	S	S	S	S	S	S	S	S
Cefuroxime	CXM	30	R	S	S	S	R	S	R	R	R	S
Ciprofloxacin	CIP	5	S	S	S	S	S	S	S	S	S	S
Cefoperazone	CPZ	75	S	S	S	S	S	S	S	S	S	S
Ceftazidime	CAZ	30	R	S	S	S	S	R	R	S	R	R
Roxithromycin	RO	30	R	S	R	R	S	S	S	S	S	S
Clarithomycin	CLR	15	S	S	R	R	S	S	R	S	R	S
Co-Trimoxazole	COT	25	R	R	R	R	S	S	R	S	R	S
Netillin	NET	30	S	S	S	S	R	S	S	R	S	S
Cefaclor	CF	30	R	S	R	R	S	S	S	S	S	S
Cefotaxime	CTX	30	R	S	S	S	R	R	R	R	R	R
Cefadroxil	CFR	30	R	S	S	S	S	R	R	S	R	R
Azithromycin	AZM	15	R	R	S	S	S	S	S	S	S	S
Ampicillin/ Cloxacillin	AX	10	R	S	S	R	S	R	R	S	R	R
Penicillin	P	10	R	S	R	R	S	S	R	S	R	S
Amikacin	AK	30	S	R	S	S	S	S	S	S	S	S
Sparfloxacin	SPX	5	S	S	R	S	S	S	S	S	S	S
Ampicillin/ Sulbactam	A/S	10/10	R	R	R	R	S	R	S	S	S	R

analysis of the isolates with their closest relatives. Based on 16S rRNA gene sequencing the strain C1 showed 97.74% similarity with the genus *Bacillus* (Phylum Firmicutes), C2 showed 91.68% similarity with genus *Pseudomonas* (Phylum Proteobacteria), C3 showed 95.81%similarity to genus *Enterobacter* (Phylum Proteobacteria), and C4 showed 100% similarity to genus *Bacillus* (Phylum Firmicutes).

In the gut bacterial isolates of F1 L6 derived from the

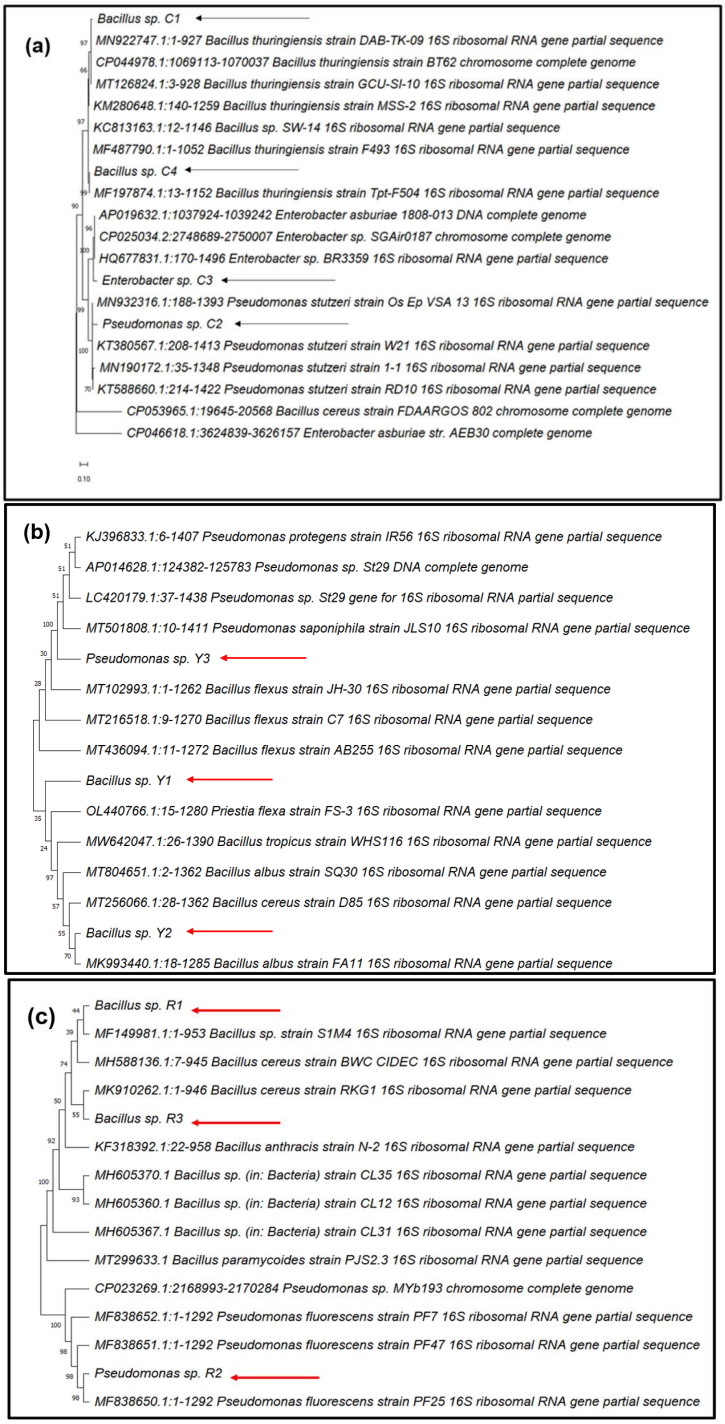

Fig. 3. Phylogenetic tree of bacteria derived from F1 L6 progeny of irradiated male parent, *S. litura* in relation to control (a) 0Gy NL6, (b) 100Gy F1L6, (c) 130Gy F1L6, using MEGA X and Neighbor joining tree (maximum likelihood method) with Bootstrap value 1000.

irradiated male parent 100Gy and 130Gy, Y1 and Y2 (100Gy regimen) showed 99.62% and 86% similarity to the genus *Bacillus*, and Y3 showed 93.06% similarity to *Pseudomonas*. Whereas R1 and R3 (130Gy regimen) showed 100% and 95.73% simlarity to *Bacillus*, and the R2 showed 93.25% similarity to *Pseudomonas*. The bacterial isolates had less similarity with already known bacterial strains in the NCBI suggesting that they could be novel bacterial isolates. Overall, it could be surmised that the bacteria in the control (0Gy) might belong to the genera *Bacillus*, *Pseudomonas,* and *Enterococcus*, whereas *Bacillus* and *Pseudomona*s were predominantly present in irradiated regimens (100Gy and 130Gy) unlike *Enterococcus* which was categorically identified in control. *Bacillus* is a Gram-positive bacteria that belong to the Phylum Firmicutes whereas *Pseudomonas* is a Gram-negative bacteria that belongs to the Phylum Proteobacteria. It is apparent from our findings that bacteria from the phylum Firmicutes and Proteobacteria were predominantly present in the Lepidopteran larval gut. The previous studies also reported that Bacillaceae and Enterobacteriaceae dominated culturable gut bacteria in the Lepidopterans. These symbionts might be contributing greatly to the physiology of these insects. A study by Hui et al. (2010) also reported that gut bacteria *Enterobacter* and *Enterococcus* isolated from lepidopteran aided in maintaining the gut pH and also enhanced the alkalinity which might be playing a critical role in tannin digestion.

In the present study, the isolated bacteria might aid in the detoxification of tannins in castor leaves (*Ricinus communis*) the primary food plant for *S. litura* which contained about 20% tannin content (Tang et al., 2012). In silkworm, *Bombyx mori* the bacteria phylum Firmicutes and Proteobacteria were reported to dominate (Liu et al., 2020). The genus *Enterococcus* was observed to be prevalent in the gut of polyphagous insects (Chen et al., 2016). The present findings corroborate earlier reports concerning these microbial groups. The bacterial isolates had less similarity with already known bacterial strains in the NCBI suggesting that they could be novel bacterial isolates. Differential behavior of the microbiota for the biochemical assays was observed in the F1 larval guts (derived from irradiated male parents) as compared to the control. Microbial CFUs titres showed that the number of cultivable bacteria were observed to be more in abundance in the irradiated regimens (100Gy F1L6, 130GyF1L6), the Genus *Enterococcus* was not identified in irradiated L6 in the present study. The bacterial abundance was found to be increased significantly due to irradiation stress, whereas the generic richness was apparently more in control than in irradiated regimens. Thus, as per the present study, the irradiation had an impact on the overall microbial diversity in the larval gut, and the observed changes in the bacterial diversity in F1 progeny of irradiated male parents might be considered as an attributing factor to induced sterility and competence of F1 moths that would be employed in IS technique for *S. litura* suppression. As only about 1% of the total bacteria were cultivable so it could not be feasible to determine the exact diversity present in the gut of the irradiated compared to the normal through the culture-dependent approach; hence, the further studies on culture independent bacteria are in progress to ascertain the bacterial diversity in irradiated Lepidopteran gut with respect to control.

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AUTHOR CONTRIBUTION STATEMENT

RKS conceived and designed research. CKS conducted experiments. CKS, KKS and PY assisted in the conducting experiments and validation of data. RKS, CKS and KKS analyzed the data and involved in writing, editing and reviewing the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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