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DIVERSITY OF CULTURABLE BACTERIA IN GUT OF WHITE GRUB *MALADERA INSANABILIS* (BRENSKE)

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ABSTRACT

***Maladera insanabilis* (Brenske) (Scarabaeidae: Coleoptera) is an economically important insect pest in agricultural and horticultural ecosystems. Digesting lignocellulolytic material has physiological and developmental benefits and requires microbial interaction for nutrient synthesis and utilization. Using a culture-dependent approach, we characterized the diversity of gut bacteria from different gut compartments of *M. insanabilis* larvae. Under aerobic culture conditions, the colonization of gut bacteria in the foregut revealed significantly higher CFU count on Nutrient agar (2.400x10^6± 0.206) followed by *Bacillus cereus* agar (2.743 x10^6± 0.147) and Nitrate agar (2.403 x10^6± 0.219) respectively. The hindgut recorded the highest CFU count of (2.780 x106± 0.031) on Thioglycolate media under anaerobic conditions. In the gut compartments of *M. insanabilis* larvae, there were eighteen culturable aerobic gut bacterial isolates belonging to phylum Bacillota and Pseudomonadota, and eight facultative anaerobic gut bacteria belonging to phylum Bacillota, and Pseudomonadota were found. The percentage abundance of the aerobic and anaerobic gut bacteria revealed that the genus *Bacillus* was the most abundant genera in the midgut (27.77%) and hindgut (25%), respectively. The foregut showed significantly higher Shannon (1.797±0.012) and Simpson (0.164±0.010) diversity for aerobic gut bacteria whereas anaerobic gut bacteria in the hindgut revealed significantly higher Shannon ((1.095±0.002) and Simpson diversity (0.257±0.010).**

Key words: *Maladera insanabilis*, 16S rRNA gene, phylogenetic analysis, aerobic bacteria, anaerobic bacteria, diversity indices, colonization, gut compartments, gut bacterial diversity, colony forming units (CFUs), fermentation chamber

Maladera is an important genera of scarab beetles with more than 500 known species causing economic damage and they are smaller than other white grub species (Bedding et al., 1983). *Maladera insanabilis* (Brenske) (Coleoptera: Scarabaeidae) is one of the most economically important species and its larvae feed on the young roots of transplants and ultimately kill the plants depending on the plant age and larval density (Pathania et al., 2015). *M. insanabilis* is commonly found in hilly districts of Himachal Pradesh (Pathania 2014). Chandel et al. (1997) recorded damage of *M. insanabilis* on apple, pear, apricot, plum and olive at Solan in Himachal Pradesh. In Jammu and Kashmir, it has been recorded as a serious pest of mulberry (Sharma and Tara 1985).

The alimentary tract of typical scarabaeid larvae comprises three main parts: a foregut, a long midgut, and a modified expanded hindgut known as the fermentation chamber (Terra, 1990; Zhang and Jackson, 2008). The anoxic fermentation chamber formed by expanding the ileum of the hindgut in the white grubs houses various microorganisms which convert complicated plant polysaccharides into substances fit for aerobic metabolism (Brune, 2006; Huang et al., 2010). The typical pattern in the behaviour of scarab larvae demonstrated that pH supports the digestive process through the gut of the scarab larva, allowing effective nutrition absorption (Terra and Ferreira, 1994; Elpidina et al., 2001; Oppert et al., 2006). The dense gut bacterial symbionts present in the scarab larvae are thought to be necessary for nitrogen digestion, sulfate absorption, and fatty acid metabolism, as well as for their hosts, who lack sterols, vitamins (Vitamin-B), key amino acids, and digestive enzymes. (Douglas, 2009; Feldhaar et al., 2007; Gil et al., 2003; Akman et al., 2002; Thomas et al., 2009). When comparing the microbial composition of all gut regions, the anterior hindgut was

characterized by an enrichment of anaerobic groups that may contribute to essential metabolic processes such as lignocellulosic material transformation, N_2 fixation, H_2, and CH_4 production (Ceja-Navarro et al., 2014). Scarabaeids are known for harboring diverse groups of bacteria in their gut region (Egert et al., 2003; Andert et al., 2010; Chouaia et al., 2019) which may be responsible for degradation of secondary metabolites of plants and may have entomopathogenic properties. These properties can be explored only after having correct identification of gut microorganisms. There is no data regarding genetic structure of gut bacteria of *M. insanabilis* anywhere in the world till now. Therefore, in present study, an attempt was made to confirm the diversity of gut bacteria of *M. insanabilis* by sequencing of mtCOI gene.

MATERIALS AND METHODS

The third instar grubs of *M. insanabilis* were collected from a farmer's field in Barot village, Mandi district (N 320,07.847′ and E 760,80.052′, 2213 masl) of Himachal Pradesh, India. The individual grubs were transferred into separate aerobic plastic jars to avoid cannibalism and were maintained with small potato tubers at a temperature of 26°C and relative humidity of 70% at the Division of Entomology, Pusa Campus, IARI, New Delhi, India. The leg sections of grubs were homogenized using a hand homogenizer, and the DNA was extracted using the CTAB method (Ellegard and Engel, 2019). Cytochrome c oxidase 1 (mtCOI) gene was amplified using gene-specific primers LCO-GGTCCTCAT AAGATATTGG and HCO- TAACTTCAGGGTGACCAAAAAATCA. The validated PCR products were outsourced to Green Genome Labs Pvt. Ltd. in India for Sanger sequencing. The grubs were starved for 24 hours before dissection to empty the gut contents. The larvae were subjected to three-step sterilization, i.e., rinsed in double-distilled water for 30 seconds, 60 seconds in 70% ethanol, and another 30 seconds in 70% ethanol. The gut was removed by dissecting the sterilized larvae with sterile micro scissors under laminar airflow. The dissected gut was divided into foregut, midgut, and hindgut, while each compartment was briefly rinsed in sterilized 0.85% NaCl before being put into a sterile 1.5 ml Eppendorf tube. The gut homogenates were serially diluted by adding 100 µl of the homogenized sample to 900 µl of 0.85% NaCl, rapidly swirling, and then dispersing 100 µl of each dilution series (10^{-8}) in triplicate series.

The serial dilutions of each gut compartment were spread-plated on five different types of media, including Nutrient Agar (NA), Tryptone Soy Agar (TSA), *Pseudomonas* Isolation Agar (PIA), *Bacillus cereus* Frankland & Frankland Agar (BCA), and Nitrate Agar. The plates were then inoculated and incubated for 24 to 48 hours at 37°C. The gut bacterial colonies were distinguished based on colour, size, and morphology. A single representative isolate of each morphotype was then purified by continuously streaking it on similar agar plates until the purity of each culture was achieved. The total number of gut bacterial isolates was determined using colony-forming units (CFU) which was calculated using mean colony counts. In 1 milliliter of the sample, the CFU represented the total number of viable cells. The facultative anaerobic gut bacteria were isolated from the foregut, midgut, and hindgut (fermentation chamber) of *M. insanabilis*. The respective gut partitions were homogenized inside an anaerobic chamber continuously flushed with CO_2 gas. After appropriate serial dilution, the gut homogenates were inoculated onto Thioglycolate medium plates and incubated at 28 ^oC in an anaerobic condition. After 72 hr, the number of colonies was counted. Pure single colonies were isolated after repeatedly streaking colonies on Petri plates containing a thioglycolate media and incubation of the Petri plates at 28^0C in a CO_2 incubator.

The pure culture isolates of single colonies were individually grown in nutrient broth for 24 hours at 37°C. The broth cultures showing turbidity were centrifuged at 10000g. The supernatant was discarded, and the pellets were resuspended in sterile water and were used for extraction of the genomic DNA using a modified CTAB technique by following the protocol as described earlier (Msango Soko et al., 2020). The purity of the extracted DNA was checked and quantified with a Nanodrop: 3300 Fluorospectometer from (Thermo Scientific in Wilmington, Delaware, USA). The genomic DNA from bacterial isolates was amplified using the 16S rRNA primers 27F- "AGAGTTTGATCCTGGCTCAG" and 1492R- "AAGGAGGTGATCCAGCCGCA." The amplicons were extracted, purified, and sent for Sangers sequencing (Green Genome Labs Pvt. Ltd., Delhi, India). The contigs of mtCOI gene sequences of scarab grub and the 16S rRNA gene sequences of the gut bacterial isolates were prepared using BioEdit, and alignment was done using the ClustalW. The Neighbor-Joining approach was used to infer the evolutionary history (Saitou and Nei, 1987). The branch lengths on the tree are shown to scale and are in the same units as the evolutionary distances used to estimate

the phylogenetic tree. MEGA11 was used to perform evolutionary analyses (Tamura et al., 2021). A bootstrap technique with 1000 replications was used to determine the support for each clade.

The data on gut bacteria isolates of *M. insanabilis* collected from Barot Valley, Himachal Pradesh, India, was subjected to diversity analysis. Shannon Weiner Index (H') (Shannon, 1948) is represented as: $H' = -\sum P_i (\ln P_i)$, Where 'P_i'=n/N, n= Number of individuals of a species in the sample, N= total number of individuals of all species in the sample, and 'ln'= the n natural logarithm. The index values in a large sample size with more than five species range from 0 to 4.5. A score of zero indicates that a single species dominates the sample, whereas a value of 4.5 shows that the population is evenly distributed among all the species. Simpson index (D) (Simpson, 1949) is expressed as DS= ∑ni (ni-1)/N (N-1) where 'DS' is the diversity index, 'ni'= number of individuals of a species in the sample, and N = total number of individuals in the sample. Higher is the value of D, lower is the diversity, and vice-versa. Aerobic and facultative anaerobic gut bacterial CFUs and the diversity analysis such as Shannon Weiner index and Simpson index were subjected to post hoc Duncan's test for individual group comparisons ($P<0.05$) through One-way analysis of variance (ANOVA) using OPSTAT (Operational Statistics) software. Further, Whittaker rank abundance was analyzed using R software.

RESULTS AND DISCUSSION

The genomic DNA of scarab beetle grub was isolated using the CTAB method by partially sequencing the mtCOI gene fragment of 640-650 bp. The aligned sequence generated from the current study was submitted to NCBI and assigned with accession number (OP080651). For BLAST analysis, the sequences were retrieved from NCBI for phylogenetic analysis using the MEGA analytical tool. The molecular identity of the white grub species from our study is confirmed as *M. insanabilis* through phylogenetic analysis (Fig. 1).

The distinct difference in enumeration counts was observed for aerobic gut bacterial isolates across different gut compartments. Enumeration of gut bacteria ranged from 1.103 (±0.045) × 10^6 in the foregut on tryptone soya agar to 2.810 (±0.311) × 10^6 in the midgut of nitrate agar. The foregut showed significantly higher CFU on nutrient agar media (2.400 (±0.206) ×10^6 CFU ml^{-1} and *B. cereus* agar (2.743 (±0.147) ×10^6 CFU ml^{-1}, followed by midgut on *Pseudomonas* agar (2.527 (±0.234) ×10^6 CFU ml^{-1}, Tryptone soya agar

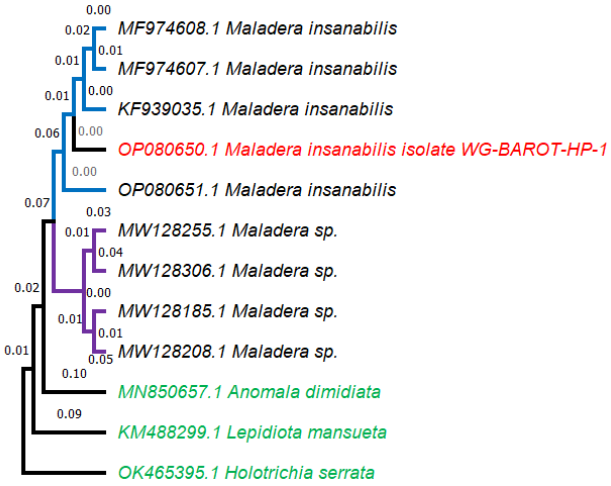

Fig. 1. Phylogenetic tree of *M. insanabilis* (Barot Valley, Himachal Pradesh, India). (Evolutionary analysis was conducted in MEGA11. The evolutionary history was inferred using the neighbor-joining method. The accession denoted with red color in the tree was obtained from our collection).

(2.587 (±0.251) ×10^6 CFU ml^{-1}, and nitrate agar (2.810 (±0.311 ×10^6 CFU ml^{-1}. In comparison, the hindgut showed lower CFUs on all four different media (Table 1). Significant differences in CFU count were observed for the facultative anaerobic gut bacterial isolates in the hindgut (2.780 (±0.031) ×10^6 CFU ml^{-1}, followed by midgut (2.130 (±0.015) ×10^6 CFU ml^{-1} and foregut 1.707 (±0.175) ×10^6 CFU ml^{-1} (Table 1). According to Msango Soko et al. (2020), the gut bacterial CFUs of *Anomala dimidiata* (Hope) showed no significant differences across the gut compartments on culturable aerobic and facultative anaerobic gut bacterial isolates, suggesting colonization of gut bacteria across the gut compartments appeared to be uniform. Zhang and Jackson (2008) carried out Scanning Electron microscopy indicating abundant distribution of gut bacteria in the fermentation chamber of *Costelytra zealandica* (White), with a bulk of them being attached to the hindgut wall.

Using a culture-dependent technique, aerobic and facultative anaerobic gut bacteria were isolated from three separate gut compartments of third-instar grubs of *M. insanabilis* (Fig. 2, 3). Generic identification of these isolates was done through 16S rRNA sequence analysis. Eight distinct aerobic bacteria colonies were isolated from the three distinct gut compartments of *M. insanabilis* larvae. *Bacillota* was the most dominant phyla with 11 bacterial isolates, followed by *Pseudomonadota* with seven bacterial isolates. The isolated aerobic bacterial strains are listed in Table 1. The genus *Bacillus* alone constituted 56% of the aerobic and 62% of the total anaerobic cultivable gut

Table 1. Colonization of aerobic and anaerobic gut bacteria in gut compartments of *M. insanabilis* larvae (Barot Valley, Himachal Pradesh, India)

Gut compartments	Culture condition: Aerobic					Culture condition: Anaerobic
	Nutrient agar	*Bacillus cereus* agar	*Pseudomonas* isolation agar	Tryptone soya agar	Nitrate agar	Thioglycolate medium
	Colony Forming Unit CFU ml^{-1} ($\times 10^6$) (Mean ± Std. Error)					
Foregut	2.400± 0.206[a]	2.743± 0.147[a]	1.737± 0.068[b]	1.103± 0.045[b]	2.403± 0.219[a]	1.707± 0.175[c]
Midgut	1.573± 0.074[b]	1.927± 0.017[b]	2.527± 0.234[a]	2.587± 0.251[a]	2.810± 0.311[a]	2.130± 0.015[b]
Hindgut	1.617± 0.104[b]	1.877± 0.038[b]	1.260± 0.192[b]	1.293± 0.098[b]	1.257± 0.039[b]	2.780± 0.031[a]
P value	0.0097	0.0007	0.0069	0.0010	0.0062	0.0009
C.D.	0.494	0.311	0.632	0.556	0.779	0.363
SE(m)	0.140	0.088	0.179	0.158	0.221	0.103
SE(d)	0.198	0.125	0.253	0.223	0.312	0.145
C.V.	13.012	6.995	16.857	16.446	17.725	8.078

C.D: Critical Difference, SE (m): Standard error of the mean, SE(d): Standard error of difference (with three replicates) CV: Coefficient of variation. Means followed by the same letter in each column were not significantly different ($p < 0.05$) by post hoc Duncan's test for individual group comparisons after One-way analysis of variance (ANOVA).

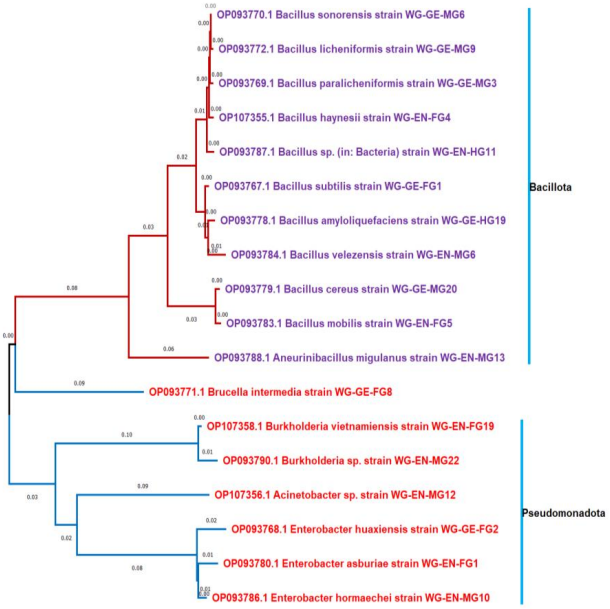

Fig. 2. Phylogenetic tree of aerobic gut bacteria isolated from *M. insanabilis* (Barot Valley, Himachal Pradesh, India). The evolutionary history inferred using Neighbor-Joining approach. Evolutionary analyses conducted in MEGA11.

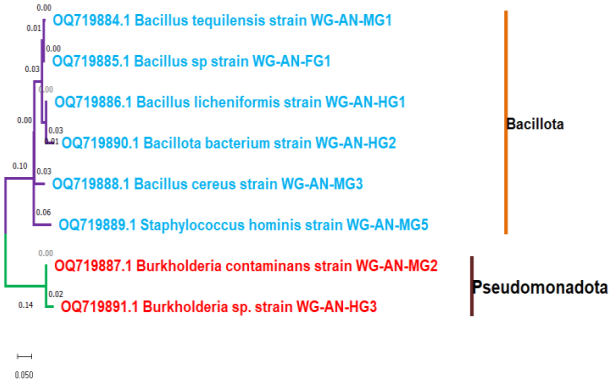

Fig. 3. Phylogenetic tree of facultative anaerobic gut bacteria isolated from *M. insanabilis* larvae (Barot Valley, Himachal Pradesh, India). Evolutionary analyses conducted in MEGA11. The evolutionary history inferred using Neighbor-Joining approach.

bacterial isolates of *M. insanabilis* (Figures 4A and B). *Enterobacter* in the foregut comprised 11%; and it was found to be is the second dominant aerobic gut bacteria. In midgut, midgut *Enterobacter* constituted only 6%. The genus *Burkholderia* is the second dominant anaerobic gut bacteria is in both the midgut and hindgut, respectively (Figure 4A and 4B). The genera of gut bacterial genera, such as *Burkholderia*, *Aneurinibacillus*, *Brucella*, and *Acinetobacter,* varied proportionately.

Earlier worker has demonstrated the colonization of a comparable cultivable bacterial community, with the dominance of γ-Proteobacteria (99.5%) in the hindgut of third-instar grubs of *Melolontha hippocastani* F (Arias-Cordero et al., 2012). However, Hernandez et al. (2015) reported the dominance of Firmicutes in the larvae of *Thorectes lusitanicus*, (Jekel) Eight distinct pure colonies of gut bacteria were isolated under anaerobic culture conditions in the present study, six isolates were found belonging to *Bacillota*, while remaining two belonged to *Pseudomonadota* (Table 2). The results obtained in the study also find support from previous studies that phylum *Proteobacteria* is the dominant group of bacteria inhabiting the gut of Scarabaeids as well as the guts of animals in general (Tamames et al., 2010).

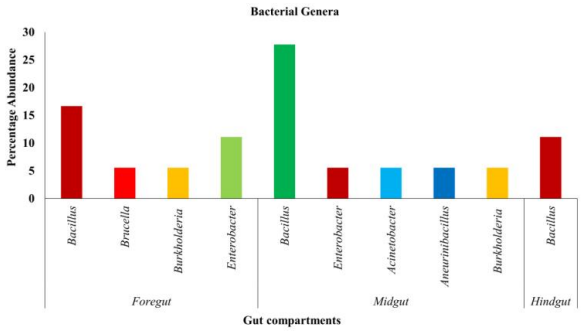

Fig. 4A. Aaerobic gut bacterial genera derived from larvae of *M. insanabilis* (Barot, Himachal Pradesh, India)

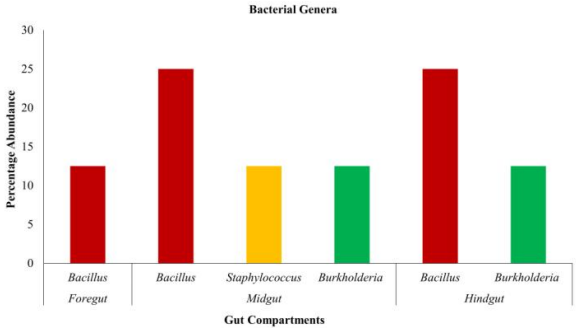

Fig. 4B. Abundance of anaerobic gut bacterial genera derived from the larvae of *M. insanabilis* (Barot, Himachal Pradesh, India)

Table 2. Culturable aerobic and anaerobic gut bacteria isolated from gut of *M. insanabilis* larvae (Barot Valley, Himachal Pradesh, India)

Strain ID	Closest relative from the gene bank	Gene Bank Accession No.	Percent similarity (%)	Phylum	Family
Aerobic gut bacteria					
WG-GE-MG6	Bacillus sonorensis	OP093770	100	Bacillota	Bacillaceae
WG-GE-MG9	*Bacillus licheniformis*	OP093772	100	Bacillota	Bacillaceae
WG-GE-MG3	*Bacillus paralicheniformis*	OP093769	100	Bacillota	Bacillaceae
WG-EN-FG4	*Bacillus haynesii*	OP107355	100	Bacillota	Bacillaceae
WG-EN-HG11	*Bacillus* sp	OP093787	100	Bacillota	Bacillaceae
WG-GE-FG1	*Bacillus subtilis*	OP093767	100	Bacillota	Bacillaceae
WG-GE-HG19	*Bacillus amyloliquifaciens*	OP093778	100	Bacillota	Bacillaceae
WG-EN-MG6	*Bacillus velezensis*	OP093784	100	Bacillota	Bacillaceae
WG-GE-MG20	*Bacillus cereus*	OP093779	100	Bacillota	Bacillaceae
WG-EN-FG5	*Bacillus mobilis*	OP093783	100	Bacillota	Bacillaceae
WG-EN-MG13	*Aneurinibacillus migulanus*	OP093788	100	Bacillota	Paenibacillaceae
WG-GE-FG8	*Brucella intermedia*	OP093771	100	Pseudomonadota	Brucellaceae
WG-EN-FG19	*Burkholderia vietnamensis*	OP107358	100	Pseudomonadota	Burkholderiaceae
WG-EN-MG22	*Burkholderia* sp	OP093790	100	Pseudomonadota	Burkholderiaceae
WG-EN-MG12	*Acinetobacter* sp	OP107356	100	Pseudomonadota	Moraxellaceae
WG-GE-FG2	*Enterobacter huaxensis*	OP093768	100	Pseudomonadota	Enterobacteriaceae
WG-EN-FG1	*Enterobacter asubriae*	OP093780	100	Pseudomonadota	Enterobacteriaceae
WG-EN-MG10	*Enterobacter hormaechi*	OP093786	100	Pseudomonadota	Enterobacteriaceae
Anaerobic gut bacteria					
WG-AN-MG1	*Bacillus tequilensis*	OQ719884	100	Bacillota	Bacillaceae
WG-AN-FG1	*Bacillus* sp	OQ719885	100	Bacillota	Bacillaceae
WG-AN-HG1	*Bacillus licheniformis*	OQ719886	100	Bacillota	Bacillaceae
WG-AN-HG2	*Bacillota bacterium*	OQ719890	100	Bacillota	Bacillaceae
WG-AN-MG3	*Bacillus cereus*	OQ719888	100	Bacillota	Bacillaceae
WG-AN-MG5	*Staphylococcus hominis*	OQ719889	100	Bacillota	Staphylococcaceae
WG-AN-MG2	*Burkholderia contaminans*	OQ719887	100	Pseudomonadota	Burkholderiaceae
WG-AN-HG3	*Burkholderia* sp	OQ719891	100	Pseudomonadota	Burkholderiaceae

Table 3. Diversity indices of aerobic and anaerobic gut bacteria of *M. insanabilis* (Barot, Himachal Pradesh, India)

Sl.No.	Gut compartments	Shannon Index (H)		(E) Evenness		Simpson Index (D)	
		Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
1	Foregut	1.797± 0.012[a]	0.896± 0.002[c]	1.637	0.816	0.164± 0.010[b]	1.000± 0.000[a]
2	Midgut	1.716± 0.009[b]	1.036± 0.009[b]	1.563	0.943	0.178± 0.019[b]	0.283± 0.005[b]
3	Hindgut	1.497± 0.008[c]	1.095± 0.002[a]	1.363	0.997	1.000± 0.000[a]	0.257± 0.010[c]
P value (0.05)		0.003	0.000			0.000	0.000
C.D.		0.222	0.020			0.045	0.022
SE(m)		0.010	0.006			0.013	0.006
SE(d)		0.014	0.008			0.018	0.009
CV		8.486	0.980			4.901	2.098

The table represents the Shannon (H) and Simpson (D), and Evenness (E) values of bacteria present inside different gut compartments of *M. insanabilis*. Shannon and Simpson's indices also indicate the most dominant species inside the gut. ± represents the standard deviation. ANOVA, standard deviation, and post hoc Duncan's test ($P<0.05$) were done using OPSTAT (Operation Statistics) software. SE (m) is the Standard error of the mean, SE (d) indicates the Standard error of difference with three replicates, and CV% indicates the coefficient of Variation.

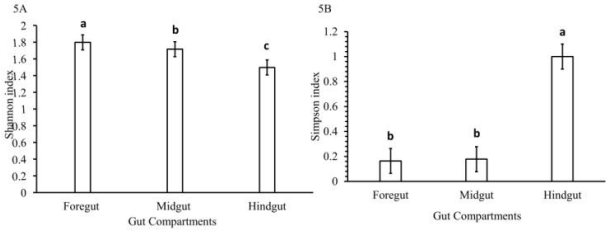

Fig. 5. Shannon and Simpson diversity indices of aerobic gut bacteria

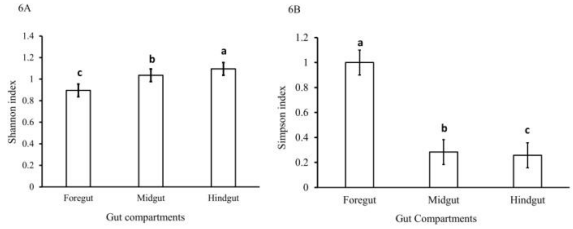

Fig. 6. Shannon and Simpson diversity indices of anaerobic gut bacteria

The diversity indices provide essential information about the rarity and prevalence of a species in a community. Shannon and Simpson indices were used to evaluate the diversity of the gut bacterial community (Fig. 5, 6). The total number of bacteria found in each gut compartment of *M. insanabilis* was used to construct the richness and diversity (H) indices. Shannon diversity index of aerobic bacteria was significantly higher in the foregut (H= 1.73; E= 1.637) compared to the midgut and hindgut (Table 3; Fig. 5), and the lowest Shannon diversity was observed in the foregut (H= 0.896; E= 0.816) (Table 3; Fig. 6). Statistical analysis of the Simpson index for aerobic gut bacteria revealed that the foregut had the lowest Simpson value (D= 0.164), indicating high diversity. For anaerobic bacteria, the Shannon index was found to be significantly higher in the hindgut (H= 1.095; E= 0.997), revealing high diversity, and the Simpson index value is highest in the foregut (D= 1.00), indicating low diversity and vice-versa **(**Table 3 and Fig. 6B**)**. The genus *Bacillus* was present in all the gut regions implying this genera's high degree of evenness in distribution. Thus, the diversity indices analysis suggests that *Bacillus* is the predominant gut bacterial genera in *M. insanabilis* across different gut compartments. (Webb et al., 2002; Horner-Devine and Bohannan, 2006), although the FG is lighter than other regions, it had the greatest bacterial diversity. Compared to other groups, the anterior hindgut was the least diverse.

The evolutionary distances were calculated for *M. insanabilis* grub using the Maximum Composite Likelihood technique and Neighbor-Joining method for aerobic and facultative anaerobic gut bacteria, and the phylogenetic trees were constructed. Two distinct clades were seen in the phylogenetic trees of aerobic and facultative anaerobic gut bacterial isolates of *M. insanabilis* (Fig. 2 and 3). The phylogenetic tree of aerobic gut bacteria shows the presence of two distinct clades; one clade belongs to the group *Bacillota* with the genus *Bacillus* (10 isolates) and *Aneurinibacillus* (1 isolate), while *Pseudomonadota* represented the other clade with the genus *Brucella* (1 isolate), *Burkholderia* (2 isolates), *Acinetobacter* sp

(1 isolate), and three isolates of *Enterobacter* (Fig. 2). For facultative anaerobic gut bacteria, *Bacillota* is the predominant gut inhabitant of *M. insanabilis*, having six isolates and *Staphylococcus* with one isolate. The phylum *Pseudomonadota* had two isolates belonging to genus *Burkholderia* (Fig. 3). According to Msango Soko et al. (2020), the phylogenetic analysis of the 16S rRNA gene sequences from culturable aerobic and facultative anaerobic gut bacteria from *A. dimidiata,* the dominant phylum was Firmicutes with *Bacillus* as the most prevailing genus, with 26 different species of aerobic bacteria and six bacterial isolates of facultative anaerobes.

The present study outlines a detailed investigation of the diversity of culturable bacteria across different gut compartments of third-instar grub of *M. insanabilis* collected from Barot Valley of Himachal Pradesh for the first time. Our study is based on a culture-dependent approach, generic identification of 16S rRNA gene, and diversity analysis has identified several gut bacterial isolates. This study will form a base for further carrying in-depth testing on physiological activities and metabolic pathways of these bacteria in degrading secondary metabolites of plants rendering them susceptible to the attack of *M. insanabilis*. Additionally, entomopathogenic properties of these microorganisms can be explored for devising suitable management strategies.

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AUTHOR CONTRIBUTION STATEMENT

S Subramanian formulated and designed the research work. Anil conducted the experiments, drafted and analysed the manuscript. N S Nysanth assisted in analysis of results. Ramesh K B and Abhishek Rana contributed in sample collection, analysis, reviewing and editing. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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