Deciphering the complex interplay between gut microbiota and crop residue breakdown in forager and hive bees (*Apis mellifera* L.)

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ABSTRACT

This study investigates *A. mellifera* gut microbiota diversity and enzymatic activities, aiming to utilize identified isolates for practical applications in sustainable crop residue management and soil health enhancement. This study sampled honey bees, analyzed gut bacterial diversity via 16S rRNA gene, and screened isolates for cellulolytic, hemicellulolytic, and pectinolytic activities, with subsequent assessment of enzymatic potential. The study reveals that cellulolytic and hemicellulolytic bacterial isolates, mainly from γ-Proteobacteria, Actinobacteria, and Firmicutes, have significant potential for crop residue management. Some genera, like *Aneurinibacillus, Bacillus, Clostridium, Enterobacter, Serratia, Stenotrophomonas, Apilactobacillus, Lysinibacillus*, and *Pseudomonas*, are very good at breaking down cellulose and hemicellulase. Notable cellulose-degrading genera include *Cedecea* (1.390 ± 0.57), *Clostridium* (1.360 ± 0.86 U/mg), *Enterobacter* (1.493 ± 1.10 U/mg), *Klebsiella* (1.380 ± 2.03 U/mg), and *Serratia* (1.402 ± 0.31 U/mg), while *Aneurinibacillus* (1.213 ± 1.12 U/mg), *Bacillus* (3.119 ± 0.55 U/mg), *Enterobacter* (1.042 ± 0.14 U/mg), *Serratia* (1.589 ± 0.05 U/mg), and *Xanthomonas* (1.156 ± 0.08 U/mg) excel in hemicellulase activity. Specific isolates with high cellulolytic and hemicellulolytic activities are identified, highlighting their potential for crop residue management. The research explores gut bacterial compartmentalization in *A. mellifera*, emphasising gut physiology's role in cellulose and hemicellulose digestion. Pectinolytic activity is observed, particularly in the Bacillaceae clade (3.229 ± 0.02), contributing to understanding the honey bee gut microbiome. The findings offer insights into microbiome diversity and enzymatic capabilities, with implications for biotechnological applications in sustainable crop residue management. The study concludes by emphasizing the need for ongoing research to uncover underlying mechanisms and ecological factors influencing gut microbiota, impacting honey bee health, colony dynamics, and advancements in crop residue management.

1. Introduction

Honey bees, particularly *Apis mellifera*, play a crucial role as global pollinators, contributing significantly to food security (Aizen et al., 2008). In addition to their ecological importance, honey bees have evolved complex social behaviors and symbiotic associations with microorganisms (Engel et al., 2016). While cellulolytic activities were initially thought to be confined to plants, bacteria, and fungi, recent evidence suggests the presence of animal cellulases, particularly in invertebrates (Linton, 2020; Gouda et al., 2024). The microbiota of honey bees is essential for breaking down complex plant polymers such as pollen, impacting diverse aspects of bee metabolism. Studies implicate gut bacteria, including (*Gilliamella*) Gamma, (*Lactobacillus*) Firm, and

Bifidobacterium, in *A. mellifera* pollen digestion (Engel et al., 2012). Furthermore, the bee's gut microbiota utilizes various pollen-derived substrates, highlighting its involvement in the complex process of pollen digestion (Kesnerova, 2017). Gut bacterial isolates from stingless honey bees, such as *Bacillus safensis* BD9, *Bacillus subtilis* BD3, *Bacillus stratosphericus* PD6, and *Bacillus amyloliquefaciens* PD9, exhibit proteolytic, lipolytic, and cellulolytic activities, underscoring their vital role in nutrient acquisition (Ngalimat et al., 2019).

As the insect gut microbiota is complex and diverse, it plays a crucial role in ecological interactions, with bacteria being the dominant group (Gurung, 2019). Symbiotic connections, estimated in up to 15 % of insects, provide essential nutrients to hosts (Douglas, 1998; Dillon and Dillon, 2004). Microbiome analysis of 21 insect groups identified 18

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bacterial phyla, including Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Tenericutes (Yun et al., 2014). In the study's context, these sentences underscore the pivotal role of complex and diverse insect gut microbiota, highlighting symbiotic connections and nutrient provision. The hindgut of termites exemplifies the intricate role of the gut microbiota in cellulose digestion (Warnecke et al., 2007). Intestinal microbes contribute to dietary toxin breakdown (Ping et al., 2007; Kikuchi et al., 2012). Gut symbionts of blood-feeding insects, like kissing bugs, stinkbugs, and termites, demonstrate nitrogen fixation and nutrient synthesis (Eichler and Schaub, 2002; Hongoh et al., 2008; Nikoh et al., 2011). Research on insect intestinal microbiota gains attention in the agricultural scientific community (Mereghetti et al., 2017).

Insects, with diverse phytophagous species, show promise for discovering cellulolytic enzymes. Reports document cellulolytic activity, including cellulase identification and cloning (Watanabe et al., 1997; Girard and Jouanin, 1999; Lee et al., 2004, 2005; Wei et al., 2006; Kim et al., 2008). Reviews on insect cellulolytic activity exist (Martin, 1983; Watanabe and Tokuda, 2001), but quantitative characterization remains limited (Cazemier et al., 1997). Polysaccharide hydrolysis is crucial for insect nutrition, especially for arthropods on plant-based diets. Global bioenergy demand rises, with biomass contributing 10 % of the energy supply (50 EJ/year), involving traditional burning and biofuels (3 EJ/year). However, enzymatic hydrolysis efficiency in biorefining faces challenges due to plant cell wall heterogeneity and recalcitrance (Cazemier et al., 1997).

Effective crop residue management is crucial for multifaceted benefits in agronomy, economics, and the environment. Locally tailored strategies enhance soil quality, water retention, and crop yields, preventing erosion, promoting root growth, and recycling nutrients. Residues reduce evaporation, prevent soil erosion, and aid water conservation, reducing irrigation needs. Economically, this approach lowers input costs, fuel consumption, and enhances yields. Environmentally, it increases soil organic matter, supports biodiversity, and mitigates climate change through improved organic carbon levels and soil carbon sequestration. A holistic approach balancing economic and environmental considerations is vital for maximizing these benefits (Kumar et al., 2023).

This study explores *A. mellifera* gut microbiota diversity and quantifies their cellulolytic, hemicellulolytic, and pectinolytic specific activities to gain insights into their role in the digestion and nutrition of bees and also to exploit the possibility of these isolates in crop residue management. Identifying gut bacterial isolates with strong enzymatic activities presents practical applications in crop residue management, potentially aiding in the breakdown of complex plant polymers and facilitating organic matter recycling. Understanding how these isolates interact with and decompose components like cellulose is essential, offering potential applications in sustainable crop residue management. The enzymatic prowess of these bacteria holds promise for enhancing organic matter decomposition, nutrient cycling, and overall soil health in agricultural practices, highlighting opportunities for bioaugmentation. This research links insect-microbe interactions with sustainable agriculture for a more resilient farming future.

2. Materials and methods

2.1. Sample collection, isolation and identification of gut bacteria

Honey bee samples (forager and hive bees) were collected from the hives maintained in the Division of Entomology, ICAR-IARI, Pusa Campus, New Delhi. The collected bees were surface sterilised with 70 % (v/v) ethanol and dissected using a sterilised microscissor under lamina flow to extract the whole digestive tract. The extracted gut is divided into the foregut, midgut, and hindgut, after which the different sections are homogenised in 0.85 % NaCl and stored at -20 ºC until further analysis for gut bacterial diversity. The gut homogenates from the various gut compartments were serially diluted and inoculated on agar plates containing bacteriological media in triplicate and kept for incubation. The colonies were purified further by streaking on corresponding media to obtain pure isolates (Gouda and Subramanian, 2022) **(Supplementary material 1).** Single colonies of pure culture isolates of the gut bacteria were grown for 24 h at 37 °C in the Nutrient broth. The pellet and supernatant were separated from the broth cultures after 24 h of growth by centrifugation at 13,000 rpm. The supernatant was discarded, and a modified cetyltrimethylammonium bromide (CTAB) method was used to extract DNA from the pellet. On an agarose gel, the extracted DNA quality was checked and quantified by using a NanoDrop: 3300 FluoroSpectrometer (Thermo Scientific, Wilmington, DE, USA). The 16S rRNA of each isolate was amplified by PCR using BioLine Master Mix and eubacterial primers 27F-(10 μM), (5′→ AGAGTTTGATCCTGGCTCAG→3) and 1492R-(10 μM), (5→ AAGGAGGTGATCCAGCCGCA→3′). Each reaction contained approximately 50 ng DNA, 25 μl Master Mix (2X) BioLine Master Mix, Takara Bio India Pvt. Ltd), and 0.5 mM each of forward and reverse primers. The following polymerase chain reaction (PCR) was performed in a thermal cycler (Bio-Rad C1000, Bio-Rad Laboratories Inc, Berkeley, CA, USA): one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min 40 s, followed by 72 °C for 10 min and 4 °C till next use. PCR products were examined by electrophoresis in a 1.2 % agarose gel, and bands were visualized by staining with ethidium bromide. The gels were run at 100 V for 1 h in TAE buffer (40 mM Tris-acetate, 1 mM ethylene-diamine-tetra-acetic acid (EDTA); pH 7.4). Gels were visualized under UV in the Gel Documentation system of Alpha ImagerTM gel imaging system (Alpha Innotech, USA). The 16 s rRNA gene sequences of gut bacterial isolates were submitted to NCBI under accession numbers MW742335 to MW742397, MW788400 to MW788404, MW916288 to MW916293, and MZ149986 to MZ149996 (Tables 1 and 2).

2.2. Screening of gut bacterial isolates for cellulolytic activity

Using carboxymethyl cellulose (CMC) as a substrate, the isolated bacterial cultures were screened for their cellulolytic activity. In short, individual isolates were inoculated on 0.25 % CMC agar media supplemented with Berg's minimal salts: NaNO₃ (2 g), MgSO₄ (0.5 g), K₂HPO₄ (0.5 g), FeSO₄ (0.1 g), CaCl₂ (0.2 g), MnSO₄ (0.02 g), CMC (2.5 g) as the substrate, and 2 % agar (pH 6.5). After that, the agar plates were incubated for 48 h at 37 °C. CMC degradation was tested according to Huang's (2012) protocol. After staining with Congo red and washing with 1 M NaCl, the cellulolytic activity was measured by observing a yellow zone around the colony. The cellulolytic index was measured as the diameter of the bacterial colony together with the hydrolytic zone divided by the diameter of the colony, as described by Delalibera (2005).

2.3. Estimation of total cellulase activity

Individual bacterial isolates with high CMCase activity were inoculated into a production medium containing (g/L): 10 g CMC, 0.2 g MgSO₄·7H₂O, 0.75 g KNO₃, 0.5 g K₂HPO₄, 0.02 g FeSO₄·7H₂O, 0.04 g CaCl₂, 2 g yeast extract, and 1 g d-glucose, pH 7.0 (Lisdiyanti et al., 2012), and incubated at 37°C for 36 h. The crude enzyme generated was collected after incubation by centrifugation at 10,000 rpm for 15 min at 4 °C. The cell-free extract was used as a crude enzyme to measure the cellulase activity.

Total cellulolytic activity was determined using the DNS (3,5-dinitrosalicylic acid) method by determining the amount of reducing sugars (Zhang et al., 2009). The quantity of glucose generated was estimated using the previously prepared glucose standard curve, and the results obtained were used to determine enzyme activity. One enzyme unit (U) was defined as the amount of enzyme-producing 1 μmol of reducing sugar measured as glucose per minute per ml of enzyme under specific assay conditions. Specific enzyme activity (U/mg) was obtained by dividing the enzyme units (U mL^{-1}) by the protein concentration (mg

Table 1

Details of 16S rRNA gene sequence identifications of gut bacterial isolates from different gut regions of forager bees of *A. mellifera*.

Isolate ID	Accession number	Species	Gut region
HAmf01	MW742335	*Aneurinibacillus aneurinilyticus* isolate HAmf01	Foregut
HAmf02	MW742336	*Aneurinibacillus migulanus* isolate HAmf02	Foregut
HAmf03	MW742337	*Bacillus acidovorans* isolate HAmf03	Hindgut
HAmf04	MW742338	*Bacillus albus* isolate HAmf04	Hindgut
HAmf05	MW742339	*Bacillus altitudinis* isolate HAmf05	Foregut
HAmf06	MW742340	*Bacillus amyloliquefaciens* isolate HAmf06	Midgut
HAmf07	MW742341	*Bacillus anthracis* isolate HAmf07	Foregut
HAmf08	MW742342	*Bacillus cereus* isolate HAmf08	Hindgut
HAmf09	MW742343	*Lysinibacillus fusiformis* isolate HAmf09	Foregut
HAmf10	MW742344	*Bacillus haynesii* isolate HAmf10	Foregut
HAmf11	MW742345	*Bacillus paramycoides* isolate HAmf11	Foregut
HAmf12	MW742346	*Bacillus pseudomycoides* isolate HAmf12	Hindgut
HAmf13	MW742347	*Bacillus subtilis* subsp. *stercoris* isolate HAmf13	Midgut
HAmf14	MW742348	*Bacillus thuringiensis* isolate HAmf14	Foregut
HAmf15	MW742349	*Bacillus toyonensis* isolate HAmf15	Midgut
HAmf16	MW742350	*Bacillus tropicus* isolate HAmf16	Foregut
HAmf17	MW788400	*Lysinibacillus* sp. isolate HAmf17	Midgut
HAmf17	MW916292	*Bacillus velezensis* isolate HAmf17	Midgut
HAmf18	MW788401	*Bacillus* sp. isolate HAmf18	Hindgut
HAmf18	MW916293	*Brevibacterium* sp. isolate HAmf18	Hindgut
HAmf19	MW742351	*Cedecea davisae* isolate HAmf19	Midgut
HAmf20	MW742352	*Clostridium argentinense* isolate HAmf20	Hindgut
HAmf21	MW742353	*Clostridium botulinum* isolate HAmf21	Foregut
HAmf22	MW742354	*Clostridium combesii* isolate HAmf22	Midgut
HAmf23	MW742355	*Clostridium scatologenes* isolate HAmf23	Foregut
HAmf24	MW742356	*Clostridium sporogenes* isolate HAmf24	Hindgut
HAmf25	MW742357	*Enterobacter asburiae* isolate HAmf25	Foregut
HAmf26	MW742358	*Enterobacter cloacae* isolate HAmf26	Midgut
HAmf27	MW742359	*Enterobacter hormaechei* isolate HAmf27	Midgut
HAmf28	MW742360	*Enterobacter ludwigii* isolate HAmf28	Foregut
HAmf29	MW742361	*Enterobacter cloacae* isolate HAmf29	Hindgut
HAmf29	MW916288	*Kocuria rosea* isolate HAmf29	Hindgut
HAmf30	MW742362	*Lysinibacillus fusiformis* isolate HAmf30	Foregut
HAmf31	MW742363	*Lysinibacillus sphaericus* isolate HAmf31	Hindgut
HAmf32	MW742364	*Ochrobactrum ciceri* isolate HAmf32	Midgut
HAmf33	MW742365	*Paenibacillus dendritiformis* isolate HAmf33	Hindgut
HAmf34	MW742366	*Pantoea agglomerans* isolate HAmf34	Hindgut
HAmf35	MW742367	*Stenotrophomonas* sp. isolate HAmf35	Foregut
HAmf36	MW742368	*Salmonella enterica* subsp. *enterica* serovar Tshiongwe isolate HAmf36	Foregut
HAmf37	MW742369	*Serratia entomophila* isolate HAmf37	Midgut
HAmf38	MW742370	*Serratia marcescens* isolate HAmf38	Hindgut
HAmf39	MW742371	*Serratia marcescens* isolate HAmf39	Foregut
HAmf40	MW742372	*Serratia nematodiphila* isolate HAmf40	Midgut
HAmf41	MW742373	*Serratia nematodiphila* isolate HAmf41	Hindgut
HAmf42	MW742374	*Serratia ureilytica* Isolate HAmf42	Midgut
HAmf43	MW742375	*Stenotrophomonas* sp. HAmf43	Foregut
HAmf44	MW742376	*Xanthomonas* sp. isolate HAmf44	Foregut
HAmf45	MZ149986	*Bacillus halotolerans* isolate HAmf45	Midgut
HAmf46	MW742388	*Klebsiella aerogenes* isolate HAmf46	Hindgut
HAmf47	MZ149990	*Bacillus vallismortis* isolate HAmf47	Midgut
HAmf48	MZ149994	*Bacillus circulans* isolate HAmf48	Hindgut

Table 2

Details of 16S rRNA gene sequence identifications of gut bacterial isolates from different gut regions of hive bees of *A. mellifera*.

Isolate ID	Accession number	Species	Gut region
HAmh01	MW742377	*Bacillus haynesii* isolate HAmh01	Hindgut
HAmh02	MW742378	*Bacillus licheniformis* isolate HAmh02	Foregut
HAmh03	MW742379	*Bacillus mojavensis* isolate HAmh03	Foregut
HAmh04	MW742380	*Bacillus nitratireducens* isolate HAmh04	Foregut
HAmh05	MW742381	*Bacillus paralicheniformis* isolate HAmh05	Hindgut
HAmh06	MW742382	*Bacillus paranthracis* isolate HAmh06	Hindgut
HAmh07	MW742383	*Bacillus sonorensis* isolate HAmh07	Midgut
HAmh08	MW742384	*Bacillus subtilis* subsp. *inaquosorum* isolate HAmh08	Midgut
HAmh09	MW742385	*Bacillus subtilis* subsp. *Stercoris* isolate HAmh09	Hindgut
HAmh10	MW742386	*Bacillus tequilensis* isolate HAmh10	Hindgut
HAmh11	MW742387	*Enterobacter asburiae* isolate HAmh11	Midgut
HAmh12	MW742388	*Enterobacter asburiae* isolate HAmh12	Midgut
HAmh13	MW742389	*Enterococcus mundtii* isolate HAmh13	Midgut
HAmh14	MW742390	*Klebsiella aerogenes* isolate HAmh14	Foregut
HAmh15	MW788402	*Lysinibacillus fusiformis* isolate HAmh15	Foregut
HAmh16	MW742391	*Lysinibacillus macroides* isolate HAmh16	Midgut
HAmh17	MW742392	*Lysinibacillus xylanilyticus* isolate HAmh17	Foregut
HAmh18	MW742393	*Lysinibacillus xylanilyticus* isolate HAmh18	Midgut
HAmh18	MW916289	*Microbacterium barkeri* isolate HAmh 18	Midgut
HAmh19	MW742394	*Pseudomonas aeruginosa* isolate HAmh19	Foregut
HAmh19	MW916290	*Microbacterium hydrocarbonoxydans* isolate HAmh 19	Foregut
HAmh20	MW742395	*Pseudomonas aeruginosa* isolate HAmh20	Hindgut
HAmh21	MW742396	*Pseudomonas aeruginosa* isolate HAmh21	Midgut
HAmh22	MW742397	*Serratia marcescens* isolate HAmh22	Foregut
HAmh23	MW788403	*Serratia* sp. isolate HAmh23	Midgut
HAmh24	MW788404	*Pseudomonas aeruginosa* isolate HAmh24	Foregut
HAmh24	MW916291	*Streptomyces* sp. isolate HAmh24	Foregut
HAmh25	MZ149987	*Bacillus velezensis* isolate HAmh25	Midgut
HAmh26	MZ149989	*Bacillus siamensis* isolate HAmh26	Hindgut
HAmh27	MZ149991	*Clostridium sporogenes* isolate HAmh27	Midgut
HAmh28	MZ149992	*Bacillus tequilensis* isolate HAmh28	Hindgut
HAmh29	MZ149993	*Bacillus amyloliquefaciens* isolate Hamh29	Hindgut
HAmh30	MZ149995	*Lactobacillus helveticus* isolate HAmh30	Foregut
HAmh31	MZ149996	*Lactobacillus alvei* isolate HAmh31	Foregut

peptone, 0.2 g/L MgSO$_4$, 1 g/L K$_2$HPO$_4$, and 15 g/L agar, pH 7.0). Following 48-hour incubation at 37 °C, xylan degradation was evaluated using Huang's (2012) protocol. Post-staining with Congo red and washing, hemicellulolytic activity was measured by observing a yellow zone around the colony, and the hemicellulolytic index was determined according to Delalibera (2005).

2.5. Estimation of hemicellulases

The active gut bacterial cultures were inoculated in 100 ml of basal medium containing peptone (1 %), KH$_2$PO$_4$ (0.15 %), NaNO$_3$ (0.2 %), NaCl (0.05 %), MgSO$_4$ (0.05 %), CaCl$_2$ (0.025 %), FeSO$_4$ (0.0001 %), ZnSO$_4$ (0.0001 %), CuSO$_4$ (0.0001 %) and birchwood xylan (1 %), at pH 7.0 and kept at 30 °C in a rotary shaker at 150 rpm. Then, the cultures were further preceded by the quantification of xylanase. Xylanase activity was measured according to the method of Saha (2002) using 0.5 ml of a 1 % (w/v) solution of oat spelt xylan incubated with 0.5 ml of the appropriately diluted culture supernatant in 50 mM acetate buffer (pH 5.0) for 30 min at 50 °C. The DNS technique was used to measure the

mL^{-1}). Total cellulase activity was determined using a method described by Zhang (2010).

2.4. Qualitative screening of gut bacterial isolates for hemicellulolytic activity

For hemicellulase assay, we followed the method given by Khan et al. (1986) with modifications. The bacterial suspensions were serially diluted (8-fold) by transferring 100 μl of the homogenised sample into 900 μl of 0.85 % saline, vortexing vigorously, after which 100 μl of each dilution (10^{-8}) was spread on a solid medium containing 0.25 % of birchwood xylan (2.5 g/L birchwood xylan, 5 g/L yeast extract, 5 g/L

released reducing sugars (Miller, 1959). Under the test conditions, one unit of xylanase activity was defined as the quantity of enzyme that liberated 1 μmol of xylose equivalent per minute.

2.6. Qualitative screening of gut bacterial isolates for pectinolytic activity

Screening for the presence of pectinolytic bacteria will be carried out following the method described by Hankin (1971), with a slight modification as follows: - Each pure bacterial culture obtained previously from culture-dependent studies will be inoculated on a mineral medium containing, per litre: KH_2PO_4, 4 g; $(NH4)_2SO_4$, 2 g; Na_2HPO_4, 6 g; $FeSO_4$ $7H_2O$, 1 mg; $MgSO_4$, 0.2 g; $CaCl_2$, 1 mg; HBO_3, 10 μg; $MnSO_4$, 10 μg; $ZnSO_4$, 70 μg; $CuSO_4$, 50 μg; MoO_3, 10 μg; Agar, 15 g; pH 7.4; containing 0.5 % pectin and 0.1 % yeast. The mineral medium was poured into Petri plates and inoculated with bacterial cultures. At 37 °C, the Petri plates were incubated for 24 h. For screening, we followed the method of Kavuthodi et al. (2015). After incubation, the plates were overlaid with iodide solution (1.0 g iodine, 5.0 g potassium iodide, and 330 ml H_2O) and kept undisturbed for 10 min. The colonies having pectinolytic activity were selected as per the size of the clear zone formation.

2.7. Estimation of pectinases

The actively grown bacterial cultures were inoculated in a medium containing citrus pectin (1 %), yeast extract (0.3 %), KH_2PO_4 (0.2 %), K_2HPO_4 (0.2 %), and KNO_3 (0.2 %) at pH 7.0, which was used for the extracellular production of an enzyme (Rehman et al., 2012). After 24 h of incubation, the fermented broth was centrifuged at 10,000 rpm for 5 min at 4 °C. Further, the cell-free filtrate (CFF) was assessed for pectinase production by performing an enzyme assay. The substrate was prepared by mixing 0.5 % (w/v) citrus pectin in 0.1 M of pH 7.5 phosphate buffer. For the pectinase reaction, 900 μl of prepared pectin substrate was added to 100 μl of crude enzyme in a test tube and incubated at 50 °C for 10 min in the water bath. To halt the reaction, each test tube was filled with 2 ml of dinitrosalicylic acid reagent (DNS) and put in a boiling water bath (92 °C) for 10 min. After cooling, the colour developed was measured using a spectrophotometer at 540 nm (Miller, 1959). The quantity of polygalacturonase required to release 1.0 μmol of galacturonic acid per minute is one unit of enzyme activity.

The media and chemicals employed in this study were sourced exclusively from Himedia Laboratories Pvt Ltd.

2.8. Protein determination

In the protein estimation of bacterial enzymes using the Bradford method (Bradford, 1976), 5–10 μl of the bacterial enzyme sample is pipetted into wells or cuvettes, and an appropriate volume of Bradford reagent is added. The mixture is thoroughly mixed, and after a 5–10 min incubation at room temperature, the absorbance is measured at 595 nm using a spectrophotometer (Bio-Rad Laboratories). Bovine serum albumin (BSA) (Bio-Rad, biorad.com) standard solutions with concentrations ranging from 0 to 1.0 mg/ml are prepared to generate a standard curve. This method relies on the binding of Coomassie Brilliant Blue G-250 dye to proteins, causing a shift in absorbance. A blank, containing only Bradford reagent, is used for baseline correction. The experiment is conducted in triplicate to enhance accuracy, and adjustments may be made based on the specific characteristics of the bacterial enzymes and experimental requirements.

2.9. Data analysis and statistics

The data underwent both descriptive analysis and analysis of variance (ANOVA) using the online statistical platform offered by the Indian Council for Agricultural Research – Indian Agricultural Statistical Research Institute (ICAR-IASRI).

3. Results

3.1. Isolation and molecular identification of gut bacterial isolates

The culturable gut bacteria associated with different gut compartments (foregut, midgut and hindgut) of adult worker bees of *A. mellifera* were isolated using different bacteriological media. Both hive and forager bees were sampled from the Bee hives maintained at the Apiary of Division of Entomology. Pure single colonies were obtained on the bacterial culture plates by streak plate technique. The pure culture isolates were designated with unique sample IDs. In total, 51 and 34 culturable bacteria were isolated respectively from the gut of forager bee and hive bees of *Apis mellifera*. DNA was extracted from each of these isolates and generic identity of these gut bacterial isolates were established using 16 s rRNA sequence characterization. The resultant 85 sequences were compared to the nearest relatives in the National Centre for Biotechnology Information (NCBI) database. The isolates' 16S rRNA gene sequences were submitted to GenBank and assigned accession numbers, as shown in Tables 1 and 2.

3.2. Screening for total cellulolytic activity

Qualitative screening revealed that 35 isolates (41.17 %) were found to be cellulase-positive among the 85 bacterial isolates (51 from foragers and 35 from hive bees) extracted from the gut of an Indian *A. mellifera* colony (Fig. 1a, Table 3). Of these, 17 isolates were found to have significantly higher cellulolytic activity, as revealed by large clear zones of >0.80 cm (Table 3). From both forager and hive bees, a majority of isolates extracted from the midgut showed the highest positive value for cellulolytic activity in qualitative assays. The cellulolytic index of the gut bacterial isolates from hive bees ranged between 0.46 cm (HAmh10) and 1.42 cm (HAmh21), while it ranged from 0.21 cm (HAmf39) to 1.37 cm (HAmf25) in the forager bees. Quantitative assays determined using DNS revealed highly significant differences ($P< 0.00001$ with Tukey's HSD at 5 %) for cellulolytic activity among the assayed gut bacterial isolates. The specific activity of cellulase was in the range of 0.388 (HAmf39) to 1.493 (HAmf25) U/mg of protein in the forager bees, while it was ranging between 0.47 (HAmh10) and 1.38 (HAmh21) U/mg of protein in the hive bees of *A. mellifera* (Fig. 2 and 3; Table 3). Accordingly, the isolates having activity greater than 1 U/mg (10 in the forager and 7 in the hive) are classified as having high enzymatic activity, and
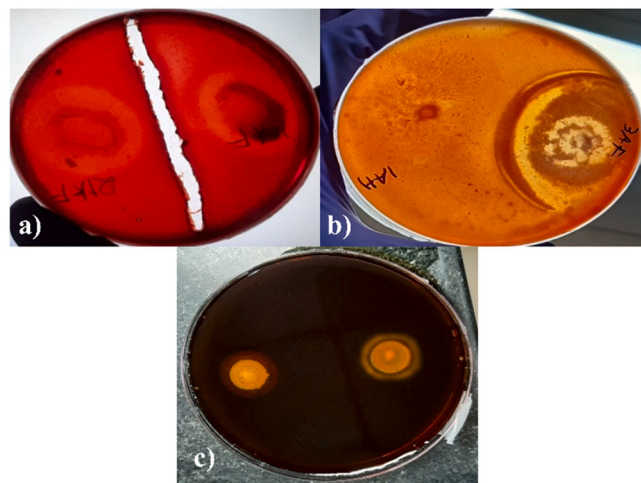

Fig. 1. Qualitative assay of isolates having positive to respective enzymatic activity (a) Degradation of CMC by *Stenotrophomonas* sp. HAmf43 and *Bacillus amyloliquefaciens* isolate HAmf06 (b) Hemicellulolytic activity in right: *Bacillus tropicus* isolate HAmf16 (c) Bacterial isolate shows the presence of pectinolytic activity in left: *Bacillus thuringiensis* isolate HAmf14 in right: *Pseudomonas aeruginosa* isolate HAmh20.

Table 3
Cellulolytic index and cellulolytic activity (mean U/mg ± SE) activity of gut bacteria from forager and hive bee of *A. mellifera*.

SL. No.	Bacteria	Gut compartment	Cellulolytic index(cm)	Specific enzyme activity (U/mg = μmol min^{-1} mg^{-1})
	Forager bee			
1	*Aneurinibacillus aneurinilyticus* isolate HAmf01	Foregut	0.68	0.757 ± 0.01
2	*Aneurinibacillus migulanus* isolate HAmf02	Foregut	0.62	0.733 ± 0.03
3	*Bacillus tropicus* isolate HAmf16	Foregut	0.56	0.782 ± 0.10
4	*Bacillus anthracis* isolate HAmf07	Foregut	0.58	0.748 ± 1.10
5	*Clostridium botulinum* isolate HAmf21	Foregut	0.67	0.750 ± 0.31
6	*Enterobacter ludwigii* isolate HAmf28	Foregut	0.68	0.750 ± 0.56
7	*Stenotrophomonas* sp. HA1f43	Foregut	0.32	0.464 ± 0.12
8	*Bacillus altitudinis* isolate HAmf05	Foregut	0.75	0.831 ± 0.03
9	*Enterobacter asburiae* isolate HAmf25	Foregut	1.37	1.493 ± 1.10
10	*Salmonella enterica* subsp. *Enterica* serovar *Tshiongwe* isolate Hamf36	Foregut	0.51	0.750 ± 0.07
11	*Serratia marcescens* isolate HAmf39	Foregut	0.21	0.388 ± 0.06
12	*Bacillus amyloliquefaciens* isolate HAmf06	Midgut	0.45	0.596 ± 0.08
13	*Bacillus subtilis* subsp. *stercoris* isolate HAmf13	Midgut	0.52	0.750 ± 1.67
14	*Clostridium combesii* isolate HAmf22	Midgut	0.92	1.360 ± 0.86
15	*Cedecea davisae* isolate HAmf19	Midgut	1.01	1.390 ± 0.57
16	*Enterobacter cloacae* isolate HAmf26	Midgut	1.13	1.369 ± 0.96
17	*Enterobacter hormaechei* isolate HAmf27	Midgut	1.10	1.290 ± 0.25
18	*Serratia ureilytica* Isolate HAmf42	Midgut	1.21	1.402 ± 0.31
19	*Serratia nematodiphila* isolate HAmf40	Midgut	1.12	1.365 ± 0.68
20	*Clostridium sporogenes* isolate HAmf24	Hindgut	0.95	1.240 ± 0.10
21	*Serratia nematodiphila* isolate HAmf41	Hindgut	1.12	1.384 ± 0.23
22	*Klebsiella aerogenes* isolate HAmf46	Hindgut	1.02	1.380 ± 2.03
	Hive bee			
23	*Klebsiella aerogenes* isolate HAmh14	Foregut	0.98	1.24 ± 0.54
24	*Serratia marcescens* isolate HAmh22	Foregut	1.11	1.27 ± 1.78
25	*Apilactobacillus helveticus* isolate HAmh30	Foregut	0.58	0.95 ± 1.95
26	*Apiactobacillus alvei* isolate HAmh31	Foregut	0.41	0.73 ± 0.36
27	*Bacillus subtilis* subsp. *inaquosorum* isolate HAmh08	Midgut	1.35	1.37 ± 0.74

Table 3 (*continued*)

SL. No.	Bacteria	Gut compartment	Cellulolytic index(cm)	Specific enzyme activity (U/mg = μmol min^{-1} mg^{-1})
28	*Lysinibacillus macroides* isolate HAmh16	Midgut	1.10	1.17 ± 0.45
29	*Pseudomonas aeruginosa* isolate HAmh21	Midgut	1.42	1.38 ± 1.20
30	*Serratia* sp. isolate HAmh23	Midgut	1.12	1.30 ± 0.91
31	*Clostridium sporogenes* isolate HAmh27	Midgut	0.98	1.17 ± 0.34
32	*Bacillus subtilis* subsp. *stercoris* isolate HAmh09	Hindgut	0.53	0.81 ± 0.22
33	*Bacillus tequilensis* isolate HAmh10	Hindgut	0.46	0.47 ± 0.35
34	*Bacillus tequilensis* isolate HAmh28	Hindgut	0.56	0.52 ± 0.07
35	*Bacillus amyloliquefaciens* isolate Hamh29	Hindgut	0.47	0.57 ± 0.12
	Mean			0.998
	p-Value			0.001
	CV			0.342
	SE(d)			0.360
	Tukeys HSD at 5 %			S

the rest of the isolates have low activity. And the seventeen isolates that have high cellulolyticactivity were also found to have higher CMC-degrading activity. Two gut bacterial isolates, *Enterobacter asburiae* (HAmf25) from the foregut and *Serratia ureilytica* (HAmf42) from the midgut, extracted from foraging bees, were found to have very high specific enzymatic activities of 1.493 U/mg and 1.402 U/mg, respectively.

3.3. Screening of hemicellulolytic gut bacteria

A positive qualitative test for hemicellulase activity is shown in Fig. 1b. The hemicellulolytic activity values ranged from 0.98 cm (*Clostridium combesii* isolate HAmf22) to 2.93 cm (*Bacillus altitudinis* isolate HAmf05) for forager bees, and they were in the range of 1.19 cm (*Microbacterium barkeri* isolate HAmh 18) to 5.33 cm (*Bacillus mojavensis* isolate HAmh03) in the case of hive bees (Table 4). Quantitative assays for hemicellulolytic activity showed highly significant differences (*P* < 0.00001 with Tukey's HSD at 5 %) between the gut bacterial isolates from both the foragers and hive bees. The specific hemicellulolytic activity (U/mg) was in the range of 0.541 (*Clostridium combesii* isolate HAmf22) to 2.083 (*Bacillus altitudinis* isolate HAmf05) in forager bees and 0.556 (*Microbacterium barkeri* isolate HAmh 18) to 3.119 (*Bacillus mojavensis* isolate HAmh03) in hive bees of *A. mellifera* (Figs. 2 and 3; Table 4). Hemicellulolytic activity was found to be predominant in the foregut of hives and forager bees, with significant differences of *P* < 0.00001 with Tukey's HSD at 5 % with respect to other gut compartments. High-significant enzymatic activities were observed in isolates like *Bacillus altitudinis* HAmf05 with 2.085 U/mg and *Bacillus mojavensis* HAmh03 with a value of 3.119 U/mg.

3.4. Screening of pectinolytic gut bacteria

Ten bacterial isolates were found to have the highest pectinolytic activity among the 50 isolates screened positive for pectinolytic activity, with the pectinolytic indices being in the range of 0.03 cm to 2.57 cm. (Fig. 1c; Table 5). Bacillus paranthracis isolate (HAmh06) showed the highest pectinolytic index (2.57 cm) in the qualitative test. The Serratia ureilytica Isolate (HAmf42), which was showing a value of 2.56 cm for the qualitative pectinolytic index, was found to show the highest pectinolytic activity of 3.693 U/mg in the quantitative test (Fig. 2). Whereas the isolate Bacillus paranthracis (HAmh06) has the highest pectinolytic
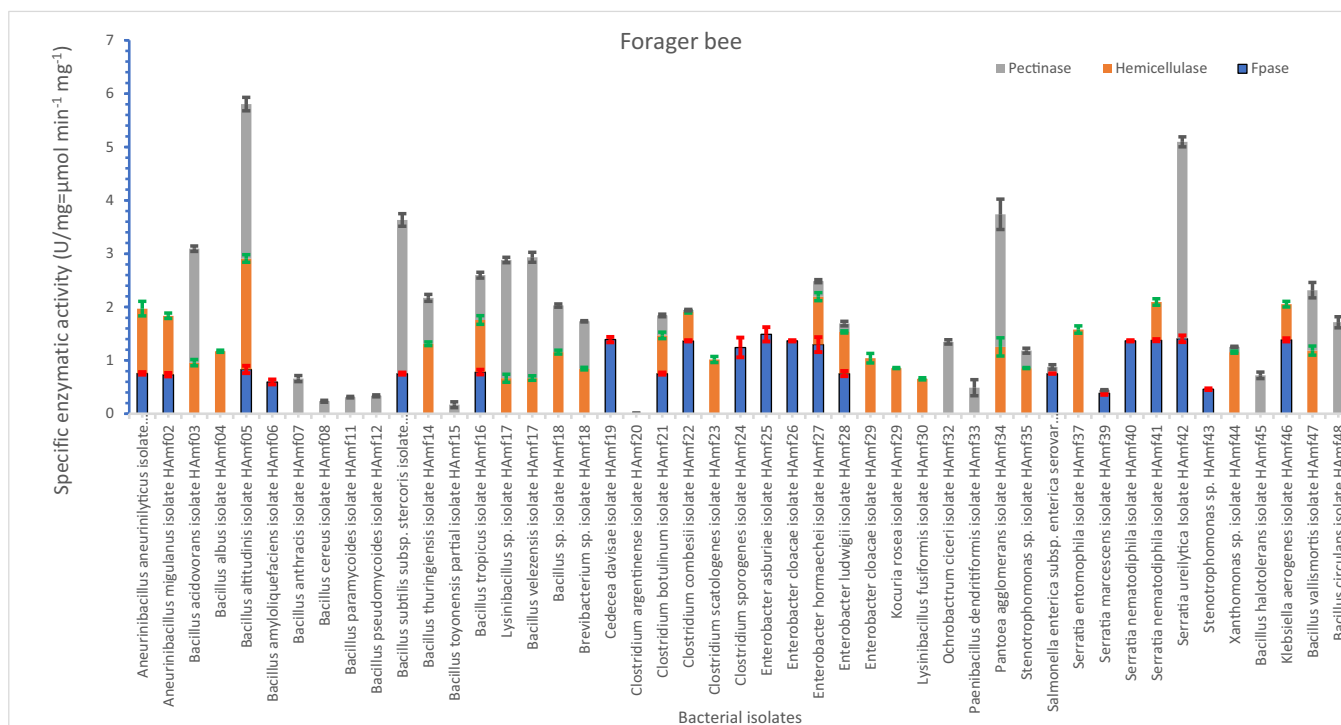
Fig. 2. Error graph showing specific enzymatic activities of gut bacterial isolates of forager bee.
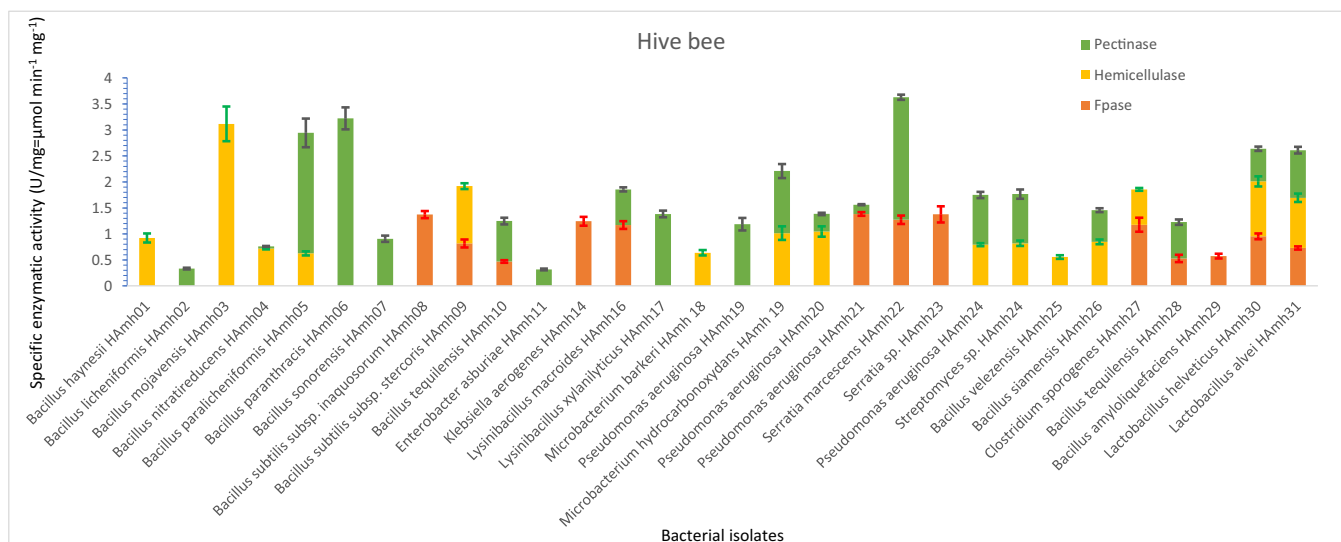

Fig. 3. Error graph showing specific enzymatic activities of gut bacterial isolates of hive bee.

index in the qualitative test and has an activity of 3.229 U/mg. And the normal specific pectinolytic activity ranged from 0.012 to 3.693 U/mg with a significant difference of $P < 0.00001$ along with Tukey's HSD at 5 % (Figs. 2 and 3; Table 5).

3.5. Phylogenetic analysis

The phylogenetic analysis of culturable gut bacteria sequences from forager bees (*Apis mellifera*) reveals a diverse composition, primarily clustered into three phyla. Firmicutes dominates with 30 isolates, predominantly represented by *Bacillus, Clostridium, Lysinibacillus, Aneurinibacillus*, and *Paenibacillus* genera. Proteobacteria follows with 19 isolates, including notable genera such as *Enterobacter, Serratia, Stenotrophomonas*, and others. Actinobacteria is the smallest phylum,

comprising 2 isolates identified as *Kocuria* and *Brevibacterium*. Among Firmicutes, *Bacillus* emerges as the dominant genus with 18 isolates. Unique bacterial phylotypes, such as *Pantoea, Aneurinibacillus, Lysinibacillus, Salmonella, Cedecea*, and *Kocuria*, were discovered. The phylogenetic tree delineates three distinct clades, offering insights into the evolutionary relationships of these gut bacteria. A detailed visual representation of this microbial diversity is depicted in **Suppl. material 2; Fig. S1,** providing a comprehensive overview of the forager bee's gut microbiota.

The gut bacteria analysis of hive bees (*Apis mellifera*) reveals a phylogenetic relationship illustrated in **Suppl. material 2; Fig. S2.** Dominated by Firmicutes (22 isolates), followed by Proteobacteria (9 isolates) and Actinobacteria (3 isolates), *Bacillus* is the prevailing genus within Firmicutes with 14 isolates. Proteobacteria includes

Table 4

Hemicellulase index and hemicellulase activity (mean U/mg ± SE) of gut bacteria from forager and hive bee of *A. mellifera*.

SL. No.	Bacteria	Gut compartment	Hemicellulase index(cm)	Specific enzyme activity (U/mg = μmol min^{-1} mg^{-1})
	Forager bee			
1	*Aneurinibacillus aneurinilyticus* isolate HAmf01	Foregut	1.73	1.213 ± 1.12
2	*Aneurinibacillus migulanus* isolate HAmf02	Foregut	1.62	1.099 ± 0.78
3	*Bacillus altitudinis* isolate HAmf05	Foregut	2.93	2.085 ± 0.36
4	*Bacillus thuringiensis* isolate HAmf14	Foregut	1.80	1.313 ± 0.01
5	*Bacillus tropicus* isolate HAmf16	Foregut	1.54	0.974 ± 0.05
6	*Clostridium botulinum* isolate HAmf21	Foregut	1.13	0.715 ± 0.04
7	*Enterobacter ludwigii* isolate HAmf28	Foregut	1.17	0.786 ± 0.06
8	*Lysinibacillus fusiformis* isolate HAmf30	Foregut	1.10	0.652 ± 0.52
9	*Stenotrophomonas* sp. isolate HAmf35	Foregut	1.27	0.857 ± 0.04
10	*Xanthomonas* sp. isolate HAmf44	Foregut	1.68	1.156 ± 0.08
11	*Bacillus velezensis* isolate HAmf17	Midgut	1.14	0.660 ± 0.58
12	*Lysinibacillus* sp. isolate HAmf17	Midgut	1.19	0.675 ± 0.49
13	*Clostridium combesii* isolate HAmf22	Midgut	0.98	0.541 ± 0.69
14	*Enterobacter hormaechei* isolate HAmf27	Midgut	1.76	0.903 ± 0.87
15	*Serratia entomophila* isolate HAmf37	Midgut	2.31	1.589 ± 0.05
16	*Bacillus halotolerans* isolate HAmf45	Midgut	1.12	0.672 ± 0.04
17	*Bacillus* vallismortis isolate HAmf47	Midgut	1.32	1.182 ± 0.09
18	*Bacillus acidovorans* isolate HAmf03	Hindgut	1.73	0.951 ± 0.07
19	*Bacillus albus* isolate HAmf04	Hindgut	1.39	1.173 ± 0.06
20	*Bacillus* sp. isolate HAmf18	Hindgut	1.70	1.156 ± 0.01
21	*Brevibacterium* sp. isolate HAmf18	Hindgut	1.65	0.840 ± 0.08
22	*Clostridium sporogenes* isolate HAmf24	Hindgut	1.20	1.014 ± 0.06
23	*Enterobacter cloacae* isolate HAmf29	Hindgut	1.47	1.042 ± 0.14
24	*Kocuria rosea* isolate HAmf29	Hindgut	1.56	0.857 ± 0.52
25	*Pantoea agglomerans* isolate HAmf34	Hindgut	1.26	1.250 ± 0.41
26	*Serratia nematodiphila* isolate HAmf41	Hindgut	1.80	0.698 ± 0.36
	Hive bee			
27	*Bacillus mojavensis* isolate HAmh03	Foregut	5.33	3.119 ± 0.55
28	*Bacillus nitratireducens* isolate HAmh04	Foregut	1.32	0.728 ± 0.98
29	*Microbacterium hydrocarbonoxydans* isolate HAmh19	Foregut	1.81	1.018 ± 0.04
30	*Pseudomonas aeruginosa* isolate HAmh19	Foregut	1.87	1.046 ± 0.07
31	*Pseudomonas aeruginosa* isolate HAmh24	Foregut	1.56	0.794 ± 0.01
32	*Streptomyces* sp. isolate HAmh24	Foregut	1.52	0.820 ± 0.08
33	*Apilactobacillus helveticus* isolate HAmh30	Foregut	1.96	1.056 ± 0.12
34	*Apilactobacillus alvei* isolate HAmh31	Foregut	2.02	0.962 ± 0.67
35	*Bacillus sonorensis* isolate HAmh07	Midgut	1.93	1.077 ± 0.87
36	*Microbacterium barkeri* isolate HAmh 18	Midgut	1.19	0.637 ± 0.34
37	*Bacillus velezensis* isolate HAmh25	Midgut	1.30	0.556 ± 1.16
38	*Clostridium sporogenes* isolate HAmh27	Midgut	0.95	0.689 ± 1.89
39	*Bacillus haynesii* isolate HAmh01	Hindgut	1.62	0.922 ± 0.05
40	*Bacillus paralicheniformis* isolate HAmh05	Hindgut	1.22	0.626 ± 0.07
41	*Bacillus subtilis* subsp. *stercoris* isolate HAmh09	Hindgut	2.01	1.102 ± 0.33
42	*Bacillus siamensis* isolate HAmh26	Hindgut	1.52	0.845 ± 0.48
	Mean			1.002
	p-Value			0.001
	CV			0.438
	SE(d)			0.335
	Tukeys HSD at 5 %			S

Table 4 (*continued*)

Pseudomonas, Enterobacter, Serratia, and *Klebsiella*. Actinobacteria comprises *Microbacterium* and *Streptomyces*. Notable novel phylotypes include *Lysinibacillus, Streptomyces*, and *Microbacterium*. The phylogenetic tree exhibits three clades, with *Bacillus subtilis* subsp. *inaquosorum* isolate HAmh08 forming a distinct clade.

The phylogenetic tree analysis (Fig. 4) of the gut bacterial isolates with cellulolytic and hemicellulolytic ability from this study revealed that cellulolytic and hemicellulolytic bacteria belonged to two phyla (Firmicutes and Proteobacteria) distributed across seven genera: Aneurinibacillus, Bacillus, Clostridium, Enterobacter, Eubacterium, Apilactobacillus, and Serratia. Whereas pectinolytic activity is additionally found in genera like Brevibacterium, Apilactobacillus, Lysinibacillus, Microbacterium, Ochrobactrum, Paenibacillus, Pantoea, Pseudomonas, Salmonella, Stenotrophomonas, Streptomyces, and Xanthomonas. The bacteria showing all three enzyme activities from forager bees are Bacillus altitudinis isolate HAmf05, Bacillus tropicus isolate HAmf16, Clostridium botulinum isolate HAmf21, Enterobacter hormaechei isolate HAmf27, and Enterobacter ludwigii isolate HAmf28. And from hive bees are Apilactobacillus helveticus isolate HAmh30 and ApiactoBacillus alvei isolate HAmh31 (Fig. 5).

4. Discussion

Cellulolytic and pectinolytic activities were analyzed in gut bacterial isolates from forager and hive bees of an Indian *Apis mellifera* colony to elucidate their role in honey bee digestion and explore their potential in crop residue management. Results indicated that cellulolytic and hemicellulolytic bacterial isolates primarily belonged to γ-Proteobacteria, Actinobacteria, and Firmicutes. Prominent cellulose-degrading genera included *Cedecea, Clostridium, Enterobacter, Klebsiella,* and *Serratia*, while

Table 5
Pectinolytic index and Pectinolytic activity (mean U/mg ± SE) of selected gut bacterial isolates from Forager and hive bee of *A. mellifera*.

SL. No.	Bacteria	Section	Pectinolytic index (cm)	Specific enzymatic activity (U/mg = µmol min^{-1} mg^{-1})
	Forager bee			
1	*Bacillus altitudinis* isolate HAmf05	Foregut	2.01	2.892 ± 0.05
2	*Bacillus anthracis* isolate HAmf07	Foregut	1.22	0.668 ± 0.08
3	*Bacillus paramycoides* isolate HAmf11	Foregut	1.13	0.312 ± 0.11
4	*Bacillus thuringiensis* isolate HAmf14	Foregut	1.96	0.865 ± 0.07
5	*Bacillus tropicus* isolate HAmf16	Foregut	1.36.	0.849 ± 0.55
6	*Clostridium botulinum* isolate HAmf21	Foregut	1.15	0.371 ± 0.02
7	*Enterobacter ludwigii* isolate HAmf28	Foregut	0.66	0.159 ± 0.69
8	*Stenotrophomonas* sp. isolate HAmf35	Foregut	0.98	0.322 ± 0.19
9	*Salmonella enterica* subsp. *enterica* serovar *Tshiongwe* isolate HAmf36	Foregut	0.38	0.125 ± 0.31
10	*Serratia marcescens* isolate HAmf39	Foregut	0.18	0.058 ± 0.58
11	*Xanthomonas* sp. isolate HAmf44	Foregut	0.30	0.098 ± 0.19
12	*Bacillus subtilis* subsp. *stercoris* isolate HAmf13	Midgut	1.98	2.880 ± 0.99
13	*Bacillus toyonensis* partial isolate HAmf15	Midgut	0.54	0.174 ± 0.14
14	*Lysinibacillus* sp. isolate HAmf17	Midgut	1.75	2.217 ± 0.75
15	*Bacillus velezensis* isolate HAmf17	Midgut	1.78	2.263 ± 0.11
16	*Clostridium combesii* isolate HAmf22	Midgut	0.11	0.030 ± 1.16
17	*Enterobacter hormaechei* isolate HAmf27	Midgut	1.15	0.288 ± 1.11
18	*Ochrobactrum ciceri* isolate HAmf32	Midgut	1.43	1.349 ± 1.05
19	*Serratia ureilytica* Isolate HAmf42	Midgut	2.56	3.693 ± 0.06
20	*Bacillus halotolerans* isolate HAmf45	Midgut	1.22	0.720 ± 0.74
21	*Bacillus vallismortis* isolate HAmf47	Midgut	1.35	1.131 ± 0.05
22	*Bacillus acidovorans* isolate HAmf03	Hindgut	1.73	2.134 ± 0.02
23	*Bacillus cereus* isolate HAmf08	Hindgut	0.82	0.236 ± 0.23
24	*Bacillus pseudomycoides* isolate HAmf12	Hindgut	1.15	0.337 ± 0.59
25	*Bacillus* sp. isolate HAmf18	Hindgut	1.20	0.874 ± 0.18
26	*Brevibacterium* sp. isolate HAmf18	Hindgut	1.23	0.886 ± 0.66
27	*Clostridium argentinense* isolate HAmf20	Hindgut	0.03	0.012 ± 0.01
28	*Paenibacillus dendritiformis* isolate HAmf33	Hindgut	1.12	*0.490 ± 0.09*
29	*Pantoea agglomerans* isolate HAmf34	Hindgut	1.82	2.487 ± 0.02
30	*Bacillus circulans* isolate HAmf48	Hindgut	1.37	1.71 ± 0.08
31	*Bacillus licheniformis* isolate HAmh02	Foregut	1.19	0.339 ± 0.01
	Hive bee			
32	*Bacillus nitratireducens* isolate HAmh04	Foregut	0.20	0.041 ± 0.04
33	*Lysinibacillus xylanilyticus* isolate HAmh17	Foregut	1.65	1.385 ± 1.10
34	*Pseudomonas aeruginosa* isolate HAmh19	Foregut	1.60	1.181 ± 0.06
35	*Microbacterium hydrocarbonoxydans* isolate HAmh 19	Foregut	1.58	1.194 ± 0.04
36	*Serratia marcescens* isolate HAmh22	Foregut	2.11	2.357 ± 0.08
37	*Pseudomonas aeruginosa* isolate HAmh24	Foregut	1.48	0.956 ± 0.01
38	*Streptomyces* sp. isolate HAmh24	Foregut	1.52	0.948 ± 0.07
39	*Apilactobacillus helveticus* isolate HAmh30	Foregut	1.29	0.627 ± 0.02
40	*Apilactobacillus alvei* isolate HAmh31	Foregut	1.46	0.919 ± 0.05
41	*Bacillus sonorensis* isolate HAmh07	Midgut	1.43	0.904 ± 0.03
42	*Enterobacter asburiae* isolate HAmh11	Midgut	1.18	0.316 ± 0.10
43	*Lysinibacillus macroides* isolate HAmh16	Midgut	1.32	0.688 ± 0.045
44	*Pseudomonas aeruginosa* isolate HAmh21	Midgut	0.90	0.177 ± 0.17
45	*Bacillus paralicheniformis* isolate HAmh05	Hindgut	2.11	2.311 ± 0.01
46	*Bacillus paranthracis* isolate HAmh06	Hindgut	2.57	3.229 ± 0.02
47	*Bacillus tequilensis* isolate HAmh10	Hindgut	1.42	0.779 ± 0.01
48	*Pseudomonas aeruginosa* isolate HAmh20	Hindgut	1.63	0.339 ± 0.70
49	*Bacillus siamensis* isolate HAmh26	Hindgut	1.27	0.616 ± 0.03
50	*Bacillus tequilensis* isolate HAmh28	Hindgut	1.32	0.705 ± 0.05
	Mean			1.021
	P-Value			0.001
	CV			0.9268
	SE(d)			1.029
	Tukeys HSD at 5 %			S

Aneurinibacillus, Bacillus, Enterobacter, Serratia, and *Xanthomonas* dominated in hemicellulase activity. Certain isolates, namely *Aneurinibacillus aneurinilyticus* HAmf01, *Bacillus altitudinis* HAmf05, *Enterobacter hormaechei* HAmf27, *Serratia nematodiphila* HAmf41 (from foragers), and *Bacillus subtilis* subsp. *stercoris* HAmh09 (from hive bees), exhibited significantly higher cellulolytic and hemicellulose activities (Figs. 6 and 7).

This research enhances our comprehension of the honeybee gut microbiota and its potential role in digestion. Within the insect orders Dictyoptera, Orthoptera, and Coleoptera, there exists a capacity for endogenous cellulase production, specifically the endo-1,4-glucanase, occurring in the midgut or salivary glands. Recent strides in insect genome sequencing have unveiled putative cellulase genes in Phthiraptera, Hemiptera, and Hymenoptera. Watanabe and Tokuda's (2010) investigation has revealed the presence of endogenous cellulases associated with glycoside hydrolase family GH9 in honey bees. Notably, many microbially produced glucoside hydrolases, including cellulases, exhibit a carbohydrate-binding module (CBM) alongside a catalytic domain. Conventionally, cellulases with CBMs are thought to remain bound to a cellulose chain post-hydrolysis, with subsequent CBM translocation to the next hydrolysis position—characteristic of processive enzymes. Processive enzymes are generally considered effective in hydrolyzing the crystalline region of cellulose. All known insect endogenous cellulases lack a CBM. However, there appears to be a
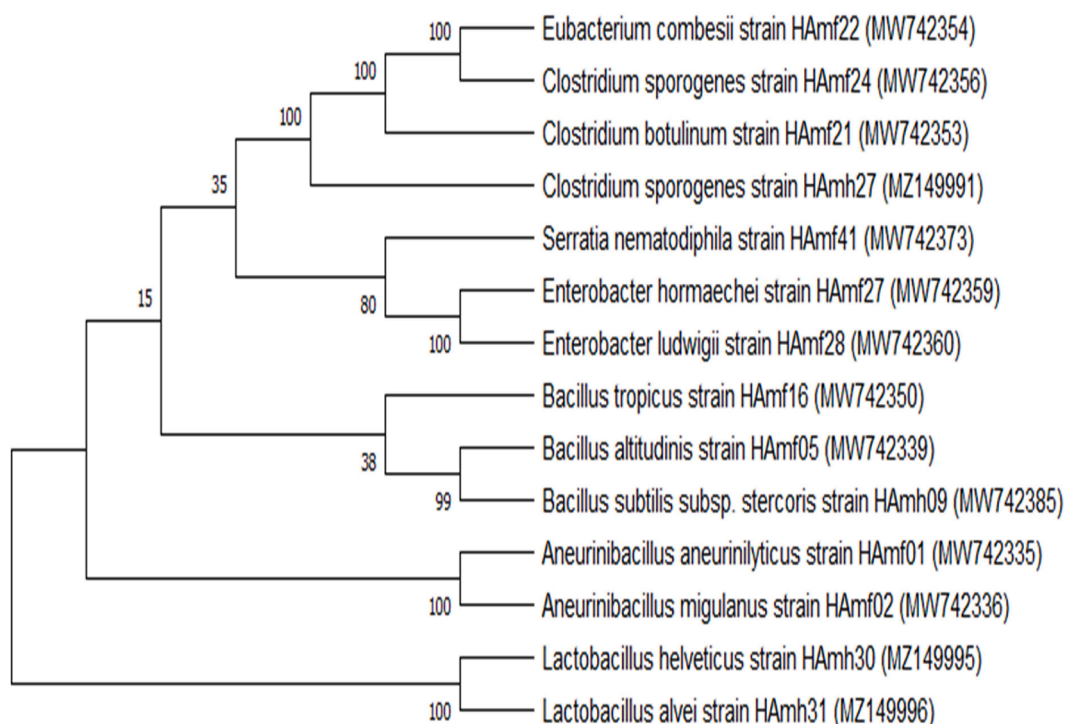
Fig. 4. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of bacterial isolates showing cellulolytic and hemicellulolytic activity in forager and hive bee of *Apis mellifera*.
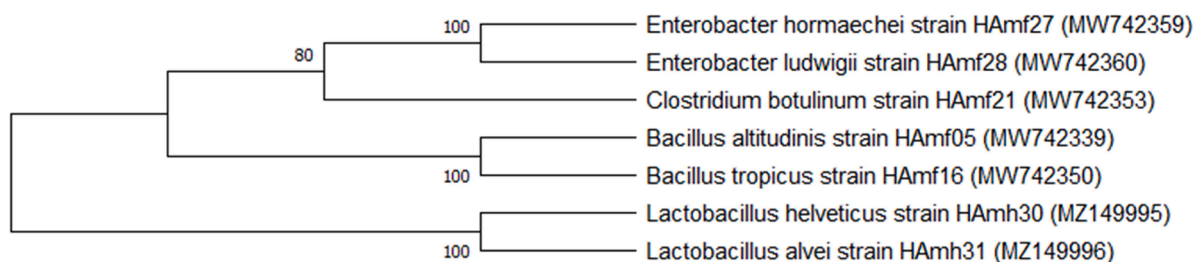

Fig. 5. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of bacterial isolates showing cellulolytic, hemicellulolytic and pectinase activity in forager and hive bee of *Apis mellifera*.

rationale for it, and bacterial cellulase compensates for its absence.

The outcomes of our investigation revealed notably elevated specific cellulolytic activities in certain isolates, such as *E. asburiae* HAmf25 (1.493 U/mg) and *Serratia ureilytica* HAmf42 (1.402 U/mg). Similarly, significant hemicellulolytic activity was observed in isolates including *Bacillus mojavensis* HAmh03 (3.119 U/mg), *Bacillus altitudinis* HAmf05 (2.085 U/mg), and *Serratia entomophila* HAmf37 (1.589 U/mg) at a level of significance of $P < 0.0001$, further validated by Tukey's Honestly Significant Difference (HSD) at 5 %. Our findings align with Lee et al. (2015), who documented that proteobacteria such as *Pseudomonas, Serratia, Enterobacter, Klebsiella,* and *Salmonella*, firmicutes like *Bacillus* and *Clostridia*, and actinobacteria isolated from *A. mellifera* possess the capability to degrade complex polysaccharides and polypeptides. Earlier research has identified high cellulolytic activity in gut bacterial isolates like *B. amyloliquefaciens, B. subtilis, E. asburiae*, and *Enterobacter cloacae* from stingless bees, specifically *Heterotrigona itama* (Ngalimat et al., 2019). Importantly, our study reinforces these earlier findings, demonstrating that these bacterial isolates exhibit significantly heightened cellulolytic activity in *A. mellifera* as well.

Genera such as *Klebsiella, Microbacterium, Bacillus, Stenotrophomonas, Pseudomonas, Enterobacter, Streptomyces*, and *Serratia* are prevalent, with prior isolations from the gastrointestinal tracts of various insect species

documented in studies by Dantur et al. (2015), Newton et al. (2013), Ngalimat et al. (2019), and Shil et al. (2014). The cellulolytic and hemicellulolytic prowess of bacterial genera such as *Bacillus paralicheniformis, B. subtilis, Pseudomonas aeruginosa, Serratia*, and *Streptomyces* has been previously established in termites (Schafer, 1996) and rhinoceros beetles (Sari et al., 2016). Consistent with these antecedent investigations, our results indicate that 22 bacterial isolates from foragers and 21 from hive bees belonging to these aforementioned genera exhibit significantly heightened cellulolytic and hemicellulolytic activities. The hemicellulolytic capacity of the bacterial genus *Microbacterium* has been demonstrated in earlier research involving mole crickets by Kim (2014). In alignment with these antecedent findings, our study identifies actinobacterial isolates, such as *Microbacterium hydrocarbonoxydans* HAmh 19 and *Microbacterium barkeri* HAmh 18 from hive bees, demonstrating significantly elevated hemicellulolytic activity. Furthermore, a *B. mojavensis* isolate from our investigation, HAmh03, displays notable hemicellulose activity (hemicellulase index 5.33 cm). This finding aligns with Kallel's (2015) report, which highlighted that a *B. mojavensis* isolate, UEB-FK, derived from the Tunisian Sahara, exhibited robust hemicellulase activity in laboratory conditions. A recent investigation conducted by Gouda et al. in 2024 has revealed that the gut bacteria of *Apis mellifera* exhibit significant proficiency in the
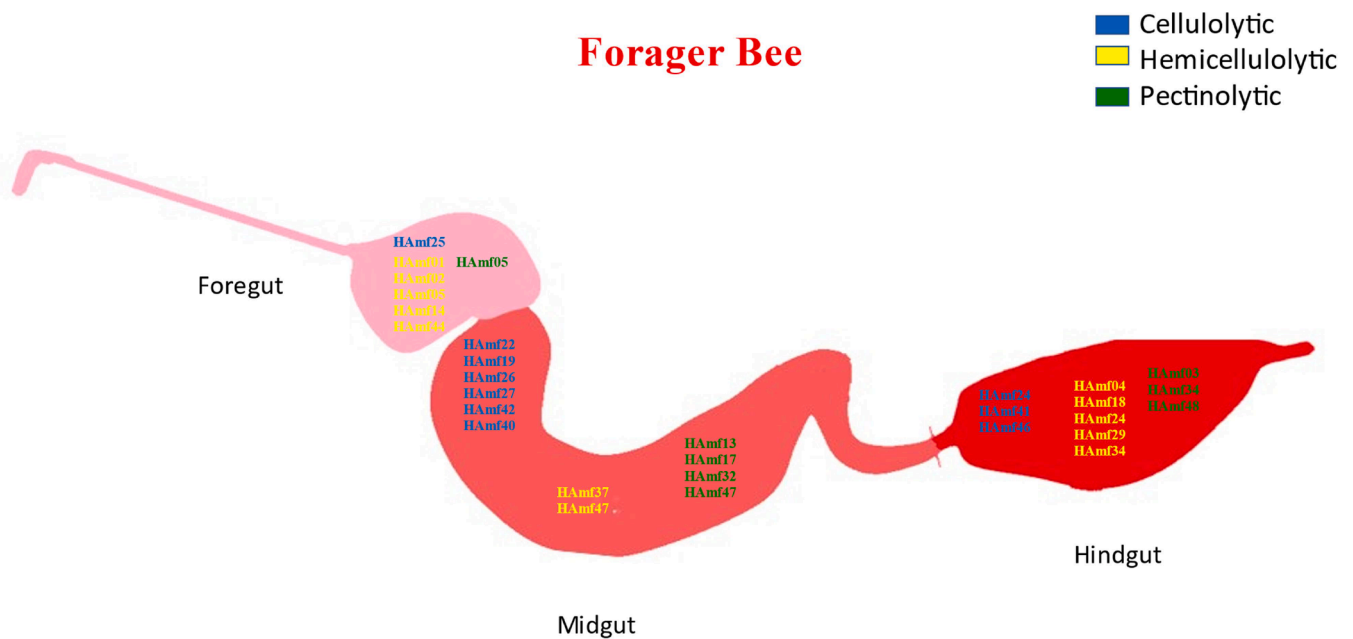
Fig. 6. Gut region of forager bee showing predominant bacterial isolates having respective enzymatic activities across different gut compartments.
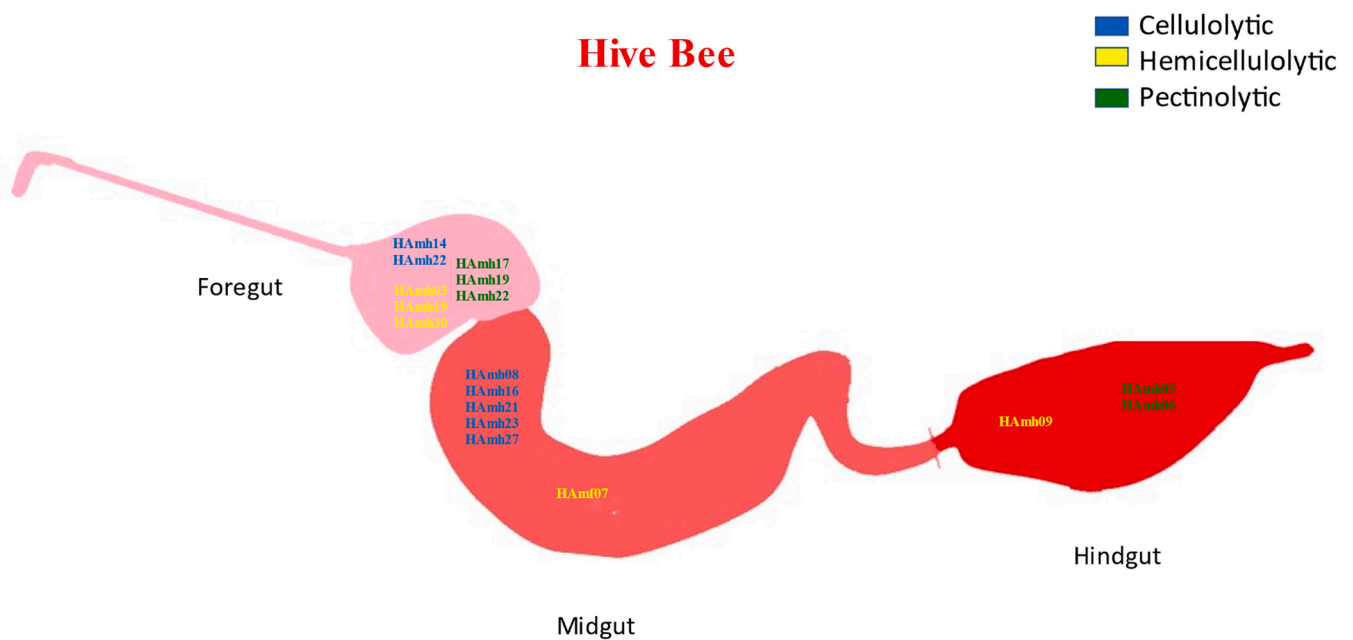

Fig. 7. Gut region of hive bee showing predominant bacterial isolates having respective enzymatic activities across different gut compartments.

degradation of various carbohydrates, including sucrose, fructose, starch, and mannose.

Gut compartmentalization emerges as a pivotal factor influencing the intricate processes of digestion and nutrient absorption within the insect gut. Distinct gut compartments, characterized by varying pH and redox conditions, orchestrate the gradual breakdown of macromolecules across different regions of the gut before their absorption in the hindgut regions of the insect. Engel et al. (2013) emphasized the significance of physicochemical variations in the insect's intestinal tract, specifying pH levels (ranging from 6 to 7 in the midgut and ileum, and maintaining at 7 in the rectum) and redox potential (fluctuating between 180 and 210 mV in the midgut and 180 to 280 mV in the hindgut). These conditions, they suggested, influence the microbial communities associated with these insects.

Pectin, a complex carbohydrate present in plant cell walls, primarily consists of galacturonic acids linked to form a gel-like substance. Its digestion requires the action of pectinolytic enzymes. Our findings highlight the notable pectinase activity within the Bacillaceae clade (Figs. 2 and 3). Parallel results from Lee et al. (2018) also identified high pectinase activity in Firmicutes and *Lactobacillus* isolated from honey bee workers. Additionally, we observed significant pectinase activity in two *Apilactobacillus* species, *A. alvei* and *A. helveticus*. Paudel (2015) documented pectinolytic activity in *Bacillus* sp. HD2 from the gut of *A. mellifera*, and Cane (2017) noted pectate lyase activity in *A. micheneri*, contributing to efficient pollen digestion and rapid protein reserve buildup in honey bees. Vuong (2019) reported pectinolytic activity in *Apilactobacillus* species in wild honey bee guts, suggesting their role in pollen digestion. In addition to the Bacillaceae, our study identified

bacterial genera from Brevibacteriaceae, Brucellaceae, Micro-bacteriaceae, Pseudomonadaceae, Streptomycetaceae, Apilactoba-cillales, and Enterobacteriaceae as having considerable pectinolytic activity in the *A. mellifera* gut. Specific isolates, such as *Serratia ureilytica* (HAmf42), *Bacillus acidovorans* (HAmf03), and *Bacillus altitudinis* (HAmf05), exhibited significantly higher pectinase activity, ranging from 0.012 to 3.693 U/mg in our study. Interestingly, forager bees displayed a significantly higher number of isolates with cellulolytic, hemicellulolytic, and pectinolytic activity compared to hive bees. This disparity may be attributed to the greater environmental exposure of forager bees compared to their hive counterparts.

The burgeoning significance and application of microbial enzymes, particularly cellulases, since the mid-1980s have paved the way for substantial biotechnological progress. Examination of the insect gut microbiome, notably in termites, has revealed the potential utilization of bacterial enzymes, specifically cellulases, across diverse industrial applications (Su et al., 2017). Certain termite groups, such as hetero-termitidae and rhinotermitidae, host highly efficient cellulolytic enzymes crucial for breaking down complex polysaccharide-rich materials, including wood (de Gonzalo et al., 2016). The hindgut of termites features a specialized cellulosome complex, comprising enzymes and structural proteins, enveloping food substrates. This complex demonstrates superior efficiency in lignocellulosic degradation compared to individual enzymes (Stern et al., 2016; Haitjema et al., 2017). The termite gut is home to a complex community of bacterial, archaeal, and eukaryotic symbionts working collaboratively to degrade plant fibers rapidly, yielding products like acetate, hydrogen, and methane (Mikaelyan et al., 2017). With the identification of over 4700 bacterial phylotypes, including Bacteroidetes, Proteobacteria, Spirochetes, Firmicutes, and Eubacteria, the termite gut microbiota plays a pivotal role in biomass degradation (Cragg et al., 2015). Similarly, cellulolytic activity has been attributed to various bacterial species from different insect orders. Examples include *Enterobacter cloacae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Pseudomonas fluorescens, Bacillus* spp., and *Mycobacterium* spp. from lepidoptera (Fischer et al., 2013; Bashir et al., 2013), *Enterococcus faecalis, Enterococcus durans, Flavobacterium odoratum, Serratia marcescens* from orthoptera (Shil et al., 2014), Firmicutes, *Clostridium* from scarabids (Engel and Moran, 2013), and *Clostridium*, Actinomycetes from termites (Gupta et al., 2011). These microbial entities contribute to biomass degradation through their cellulolytic activities.

Therefore, isolates identified in our study, such as *E. asburiae* HAmf25, *Serratia ureilytica* HAmf42, *Bacillus mojavensis* HAmh03, *Bacillus altitudinis* HAmf05, *Serratia entomophila* HAmf37, and *Bacillus altitudinis* HAmf05, exhibit noteworthy cellulase, hemicellulase, and pectinase activities. Consequently, these isolates hold potential for application in crop residue management. In essence, the comprehensive understanding of the *A. mellifera* gut microbiome and its enzymatic capabilities offers valuable insights for leveraging these processes in biotechnological applications. This progress contributes to the advancement of sustainable and eco-friendly technologies in the domain of biomass degradation.

In conclusion, our study enhances the current understanding of cellulolytic and pectinolytic activities in the gut bacteria of honey bees. The identified bacterial isolates and their enzymatic capabilities underscore the rich microbial diversity within the honey bee gut, shedding light on their potential roles in digestion and nutrition. However, it is crucial to interpret these conclusions cautiously and restrict them to the supportive evidence presented. Further investigations are imperative to delve into the intricate mechanisms governing these enzymatic activities, the ecological determinants influencing gut microbiota composition, and the repercussions of gut bacteria on honey bee health and overall colony dynamics. A more comprehensive comprehension of the honey bee gut microbiome can pave the way for developing strategies, potentially employing these predominant isolates in the realm of crop residue management.

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During the preparation of this work the author(s) used Chat GPT Open AI in order to improve language editing. After using this tool/ service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Ethics approval

This study deals with honeybees; as this is an observational study, it does not require ethical approval.

Consent to participate

Not applicable. This study does not involve human subjects.

Consent to publish

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CRediT authorship contribution statement

M.N. Rudra Gouda: Conceptualization, Methodology, Writing – original draft, Software, Data curation, Visualization, Investigation, Writing – review & editing. **K.M. Kumaranag:** Writing – review & editing. **B. Ramakrishnan:** Conceptualization, Writing – review & editing. **Sabtharishi Subramanian:** Conceptualization, Methodology, Data curation, Visualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data generated during this study are included in this article [and its supplementary information files].

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2024.100233.

References

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.M., 2008. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18 (20), 1572–1575. https://doi.org/10.1016/j.cub.2008.08.066.

Bashir, Z., Kondapalli, V.K., Adlakha, N., Sharma, A., Bhatnagar, R.K., Chandel, G., Yazdani, S.S., 2013. Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts. Sci. Rep. 3 (1), 2558.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Cane, J.H., Dobson, H.E., Boyer, B., 2017. Timing and size of daily pollen meals eaten by adult females of a solitary bee (Nomia melanderi) (Apiformes: halictidae). Apidologie (Celle) 48, 17–30. https://doi.org/10.1007/s13592-016-0444-8.

Cazemier, A.E., Op den Camp, H.J., Hackstein, J.H., Vogels, G.D., 1997. Fibre digestion in arthropods. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol. 118, 101–109.

Cragg, S.M., Beckham, G.T., Bruce, N.C., Bugg, T.D.H., Distel, D.L., Dupree, P., Zimmer, M., 2015. Lignocellulose degradation mechanisms across the tree of life. Curr. Opin. Chem. Biol. 29, 108–119.

Dantur, K.I., Enrique, R., Welin, B., Castagnaro, A.P., 2015. Isolation of cellulolytic bacteria from the intestine of Diatraea saccharalis larvae and evaluation of their capacity to degrade sugarcane biomass. AMB Express 5 (1), 1–11. https://doi.org/10.1186/s13568-015-0101-z.

De Gonzalo, G., Colpa, D.I., Habib, M.H., Fraaije, M.W., 2016. Bacterial enzymes involved in lignin degradation. J. Biotechnol. 236, 110–119.

Delalibera Jr, I., Handelsman, J.O., Raffa, K.F., 2005. Contrasts in cellulolytic activities of gut microorganisms between the wood wasp, Saperda vestita (Coleoptera: cerambycidae), and the bark beetles, Ips pini and Dendroctonus frontalis (Coleoptera: curculionidae). Environ. Entomol. 34 (3), 541–547. https://doi.org/10.1603/0046-225X-34.3.541.

Dillon, R.J., Dillon, V.M., 2004. The gut bacteria of insects: nonpathogenic interactions. Annu. Rev. Entomol. 49 (1), 71–92. https://doi.org/10.1146/annurev.ento.49.061802.123416.

Douglas, A.E., 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria Buchnera. Annu. Rev. Entomol. 43 (1), 17–37. https://doi.org/10.1146/annurev.ento.43.1.17.

Eichler, S., Schaub, G.A., 2002. Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. Exp. Parasitol. 100 (1), 17–27. https://doi.org/10.1006/expr.2001.4653.

Engel, P., Kwong, W.K., McFrederick, Q., Anderson, K.E., Barribeau, S.M., Chandler, J.A., Emery, O., 2016. The bee microbiome: impact on bee health and model for evolution and ecology of host-microbe interactions. MBio 7 (2), e02164. https://doi.org/10.1128/mBio.02164-15. -15.

Engel, P., Martinson, V.G., Moran, N.A., 2012. Functional diversity within the simple gut microbiota of the honey bee. Proc. Natl Acad. Sci. 109 (27), 11002–11007. https://doi.org/10.1073/pnas.1202970109.

Engel, P., Moran, N.A., 2013. The gut microbiota of insects – diversity in structure and function. FEMS Microbiol. Rev. 37 (5), 699–735. https://doi.org/10.1111/1574-6976.12025.

Fischer, R., Ostafe, R., Twyman, R.M., 2013. Cellulases from insects. Yellow Biotechnol. II: Insect Biotechnol. Plant Prot. Ind. 51–64.

Girard, C., Jouanin, L., 1999. Molecular cloning of cDNAs encoding a range of digestive enzymes from a phytophagous beetle, Phaedon cochleariae. Insect Biochem. Mol. Biol. 29, 1129–1142.

Gouda, M.R., Subramanian, S., 2022. Studies on the culturing and isolation of gut bacteria of Apis mellifera L. Pharma Innov. J. 726–728.

Gouda, M.R., Subramanian, S., Kumar, A., Ramakrishnan, B., 2024. Microbial ensemble in the hives: deciphering the intricate gut ecosystem of hive and forager bees of Apis mellifera. Mol. Biol. Rep. 51 (1), 262.

Gupta, R., Khasa, Y.P., Kuhad, R.C., 2011. Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. Carbohydr. Polym. 84 (3), 1103–1109.

Gurung, K., Wertheim, B., Falcao Salles, J., 2019. The microbiome of pest insects: it is not just bacteria. Entomol. Exp. Appl. 167 (3), 156–170. https://doi.org/10.1111/eea.12768.

Haitjema, C.H., Gilmore, S.P., Henske, J.K., Solomon, K.V., O`Malley, M.A., 2017. A parts list for fungal cellulosomes revealed by comparative genomics. Nat. Microbiol. 2, 17087. https://doi.org/10.1038/nmicrobiol.2017.87.

Hankin, L., Zucker, M., Sands, D.C., 1971. Improved solid medium for the detection and enumeration of pectolytic bacteria. Appl. Microbiol. 22 (2), 205–209. https://doi.org/10.1128/am.22.2.205-209.1971.

… & Hongoh, Y., Sharma, V.K., Prakash, T., Grigoriev, I.V., 2008. Genome of an endosymbiont coupling N2 fixation to cellulolysis within protist cells in termite gut. Science 322 (5904), 1108–1109. https://doi.org/10.1126/science.1165578.

Huang, S., Sheng, P., Zhang, H., 2012. Isolation and identification of cellulolytic bacteria from the gut of Holotrichia parallela larvae (Coleoptera: scarabaeidae). Int. J. Mol. Sci. 13 (3), 2563–2577. https://doi.org/10.3390/ijms13032563.

Kallel, F., Driss, D., Chaabouni, S.E., Ghorbel, R., 2015. Biological activities of xylooligosaccharides generated from garlic straw xylan by purified xylanase from Bacillus mojavensis UEB-FK. Appl. Biochem. Biotechnol. 175, 950–964. https://doi.org/10.1007/s12010-014-1308-1.

Kavuthodi, B., Thomas, S.K., Sebastian, D., 2015. Co-production of pectinase and biosurfactant by the newly isolated strain Bacillus subtilis BKDS1. Br Microbiol. Res. J. 10 (2), 1–2.

… & Kešnerová, L., Mars, R.A., Ellegaard, K.M., Troilo, M., Engel, P., 2017. Disentangling metabolic functions of bacteria in the honey bee gut. PLoS Biol. 15 (12), e2003467 https://doi.org/10.1371/journal.pbio.2003467.

Khan, A.W., Tremblay, D., LeDuy, A., 1986. Assay of xylanase and xylosidase activities in bacterial and fungal cultures. Enzyme Microb. Technol. 8 (6), 373–377. https://doi.org/10.1016/0141-0229(86)90139-0.

… & Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Fukatsu, T., 2012. Symbiont-mediated insecticide resistance. Proc. Natl Acad. Sci. 109 (22), 8618–8622. https://doi.org/10.1073/pnas.1200231109.

… & Kim, D.Y., Shin, D.H., Jung, S., Park, H.Y., 2014. Novel alkali-tolerant GH10 endo-β-1, 4-xylanase with broad substrate specificity from Microbacterium trichothecenolyticum HY-17, a gut bacterium of the mole cricket Gryllotalpa orientalis. J. Microbiol. Biotechnol. 24 (7), 943–953. https://doi.org/10.4014/jmb.1405.05032.

… & Kim, N., Choo, Y.M., Lee, K.S., Jin, B.R., 2008. Molecular cloning and characterization of a glycosyl hydrolase family 9 cellulase distributed throughout the digestive tract of the cricket Teleogryllus emma. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol. 150, 368–376.

Kumar, N., Chaudhary, A., Ahlawat, O.P., Naorem, A., Upadhyay, G., Chhokar, R.S., Singh, G.P., 2023. Crop residue management challenges, opportunities and way forward for sustainable food-energy security in India: a review. Soil Tillage Res. 228, 105641.

Lee, F.J., Miller, K.I., McKinlay, J.B., Newton, I.L.G., 2018. Differential carbohydrate utilization and organic acid production by honey bee symbionts. FEMS Microbiol. Ecol. 94 (8), fiy113. https://doi.org/10.1093/femsec/fiy113.

Lee, F.J., Rusch, D.B., Stewart, F.J., Mattila, H.R., Newton, I.L., 2015. Saccharide breakdown and fermentation by the honey bee gut microbiome. Environ. Microbiol. 17 (3), 796–815. https://doi.org/10.1111/1462-2920.12526.

… & Lee, S., Kim, S.R., Yoon, H.J., Kim, I., Lee, K.S., Je, Y.H., Jin, B.R., 2004. cDNA cloning, expression, and enzymatic activity of a cellulase from the mulberry longicorn beetle, Apriona germari. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol. 139, 107–116.

… & Lee, S.J., Lee, K.S., Kim, S.R., Gui, Z.Z., Kim, Y.S., Yoon, H.J., Jin, B.R., 2005. A novel cellulase gene from the mulberry longicorn beetle, Apriona germari: gene structure, expression, and enzymatic activity. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol 140, 551–560.

Linton, S.M., 2020. The structure and function of cellulase (endo-β-1, 4-glucanase) and hemicellulase (β-1, 3-glucanase and endo-β-1, 4-mannase) enzymes in invertebrates that consume materials ranging from microbes, algae to leaf litter. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol 240, 110354.

Lisdiyanti, P., Suyanto, E., Gusmawati, N.F., Rahayu, W., 2012. Isolation and characterization of cellulase produced by cellulolytic bacteria from peat soil of Ogan Komering Ilir, South Sumatera. Int. J. Environ. Bioenergy 3 (3), 145–153.

Martin, M.M., 1983. Cellulose digestion in insects. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol. 75, 313–324.

Mereghetti, V., Chouaia, B., Montagna, M., 2017. New insights into the microbiota of moth pests. Int. J. Mol. Sci. 18 (11), 2450. https://doi.org/10.3390/ijms18112450.

Mikaelyan, A., Meuser, K., Brune, A., 2017. Microenvironmental heterogeneity of gut compartments drives bacterial community structure in wood- and humus-feeding higher termites. FEMS Microbiol. Ecol. 93, fiw210. https://doi.org/10.1093/femsec/fiw210.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31 (3), 426–428. https://doi.org/10.1021/ac60147a030.

Newton, D.F., Macfarlane, S., Macfarlane, G.T., 2013. Effects of antibiotics on bacterial species composition and metabolic activities in chemostats containing defined populations of human gut microorganisms. Antimicrob. Agents Chemother. 57 (5), 2016–2025. https://doi.org/10.1128/AAC.00079-13.

Ngalimat, M.S., Rahman, R.N., Yusof, M.T., Syahir, A., Sabri, S., 2019. Characterisation of bacteria isolated from the stingless bee, Heterotrigona itama, honey, bee bread, and propolis. PeerJ 7, e7478. https://doi.org/10.7717/peerj.7478.

Nikoh, N., Hosokawa, T., Oshima, K., Hattori, M., Fukatsu, T., 2011. Reductive evolution of bacterial genome in insect gut environment. Genome Biol. Evol. 3, 702–714. https://doi.org/10.1093/gbe/evr064.

Paudel, Y.P., Lin, C., Shen, Z., Qin, W., 2015. Characterization of pectin-depolymerizing exo-polygalacturonase by Bacillus sp. HD2 isolated from the gut of Apis mellifera L. Microbiol. Discov. 3 (1), 1–8.

Ping, L., Büchler, R., Mithöfer, A., et al., 2007. A novel Dps-type protein from insect gut bacteria catalyses hydrolysis and synthesis of N-acyl amino acids. Environ. Microbiol. 9 (6), 1572–1583. https://doi.org/10.1111/j.1462-2920.2007.01279.x.

Rehman, H.U., Qader, S.A., Aman, A., 2012. Polygalacturonase: production of pectin-depolymerizing enzyme from Bacillus licheniformis KIBGE IB-21. Carbohydr. Polym. 90 (1), 387–391. https://doi.org/10.1016/j.carbpol.2012.05.055.

Saha, B.C., 2002. Production, purification, and properties of xylanase from a newly isolated Fusarium proliferatum. Process Biochem. 37 (11), 1279–1284. https://doi.org/10.1016/S0032-9592(02)00012-2.

… & Sari, S.L., Pangastuti, A., Susilowati, A., Purwoko, T., Mahajoeno, E., Hidayat, W., Anitasari, R., 2016. Cellulolytic and hemicellulolytic bacteria from the gut of Oryctes rhinoceros larvae. Biodiversitas J. Biol. Divers. 17 (1) https://doi.org/10.13057/biodiv/d170111.

Schäfer, A., Konrad, R., Kuhnigk, T., Kämpfer, P., Hertel, H., König, H., 1996. Hemicellulose-degrading bacteria and yeasts from the termite gut. J. Appl. Microbiol. 80 (5), 471–478. https://doi.org/10.1111/j.1365-2672.1996.tb03245.x.

Shil, R.K., Mojumder, S., Sadida, F.F., Uddin, M., Sikdar, D., 2014. Isolation and identification of cellulolytic bacteria from the gut of three phytophagous insect species. Braz. Arch. Biol. Technol. 57, 927–932. https://doi.org/10.1590/S1516-891320140262.

Stern, J., Moraïs, S., Lamed, R., Bayer, E.A., 2016. Adaptor scaffoldins: an original strategy for extended designer cellulosomes, inspired from nature. MBio 7 (2), e00083. https://doi.org/10.1128/mBio.00083-16.

Su, L.H., Zhao, S., Jiang, S.X., Liao, X.Z., Duan, C.J., Feng, J.X., 2017. Cellulase with high β-glucosidase activity by Penicillium oxalicum under solid-state fermentation and its use in hydrolysis of cassava residue. World J. Microbiol. Biotechnol. 33 (2), 37.

Vuong, H., 2019. Function and Transmission of Novel Wild Bee Symbionts from the Apilactobacillus micheneri Clade. University of California.

Warnecke, F., Luginbühl, P., Ivanova, N., et al., 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 450 (7169), 560–565. https://doi.org/10.1038/nature06269.

Watanabe, H., Tokuda, G., 2010. Cellulolytic systems in insects. Annu. Rev. Entomol. 55, 609–632.

Watanabe, H., Nakamura, M., Tokuda, G., Yamaoka, I., Scrivener, A.M., Noda, H., 1997. Site of secretion and properties of endogenous endo-beta-1, 4-glucanase components from Reticulitermes speratus (Kolbe), a Japanese subterranean termite. Insect Biochem. Mol. Biol. 27, 305–3013.

Watanabe, H., Tokuda, G., 2001. Animal cellulases. Cell. Mol. Life Sci. 58, 1167–1178.

... & Wei, Y.D., Lee, K.S., Gui, Z.Z., Yoon, H.J., Kim, I., Zhang, G.Z., Jin, B.R., 2006. Molecular cloning, expression, and enzymatic activity of a novel endogenous cellulase from the mulberry longicorn beetle, Apriona germari. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol 145, 220–229.

... & Yun, J.H., Roh, S.W., Whon, T.W., Jung, M.J., Kim, M.S., Park, D.S., Kim, J.Y., 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Appl. Environ. Microbiol. 80 (17), 5254–5264. https://doi.org/10.1128/AEM.01226-14.

Zhang, M., Su, R., Qi, W., He, Z., 2010. Enhanced enzymatic hydrolysis of lignocellulose by optimizing enzyme complexes. Appl. Biochem. Biotechnol. 60, 1407–1414. https://doi.org/10.1007/s12010-009-8602-3.

Zhang, Y.P., Hong, J., Ye, X., 2009. Cellulase assays. Biofuels: Methods and Protocols, pp. 213–231.