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Antimicrobial Potential of Gut-Associated Bacteria Isolated from the German Cockroach (Blattella germanica) Against Multidrug-Resistant Human Pathogens

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Abstract

The insect gut harbors complex microbial communities that play fundamental roles in host immunity and defense by producing antimicrobial substances that compete with pathogenic microorganisms. In this study, gut bacteria associated with *Blattella germanica* adults. were isolated and identified to evaluate their antimicrobial activity against multidrug-resistant (MDR) human pathogens. Seven gut bacterial species were identified morphologically and biochemically as *Pseudomonas* sp. *strain AYA1, P. mohnii, Staphylococcus sciuri, S. saprophyticus, Paenibacillus* sp., *Proteus* sp., and *Micrococcus luteus*. Their antagonistic activities were evaluated against a panel of MDR pathogens; Gram-positive bacteria (*S. aureus* and *Bacillus cereus*), Gram-negative (*Escherichia coli* and *Salmonella enteritidis*), and the fungus (*Candida albicans*). Among the isolated bacteria, *Pseudomonas* sp. *strain AYA1* exhibited antagonistic activity against the tested MDR pathogens. Followed by *P. mohnii*, which inhibited all MDR pathogens except *S. enteritidis* and *B. cereus*. While the remaining isolates showed no significant antagonistic activity against all tested MDR pathogens. Subsequently, the two active isolates were identified molecularly using 16S rRNA. These findings suggested that cockroaches' gut microbiota may contribute to the development of novel therapeutic agents against MDR pathogens. To the best of our knowledge, this study represents the first report of the isolation of *P. mohnii* from cockroaches.

Introduction

The insect gut is a specialized niche for bacterial colonization, and its microbes confer various benefits on their hosts [1, 2]. Bacteria are the dominant microbial group, mainly Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, and community composition varies by gut region [3, 4]. *B. germanica* is one of the most widespread and resilient human-associated pest insects, with a dynamic gut microbiome [5]. Bacterial abundance differs among its foregut, midgut, and hindgut, being highest in the foregut, followed by the midgut and hindgut [6, 7]. The foregut is commonly colonized by *Pseudomonas*, *Serratia*, and *Bacillus* [1, 7 and 8]. The midgut harbors *Enterococcus*, *Lactobacillus*, *Pseudomonas*, *Serratia*, *Klebsiella*, and *Enterobacter* [9]. The hindgut is dominated by Porphyromonadaceae, Rikenellaceae, Lachnospiraceae, and Ruminococcaceae [10, 11]. Gut microbiota play crucial roles in host nutrition, growth, development, immune regulation, detoxification, and vitamin synthesis[12, 13 and 14]. In *B. germanica*, *Pseudomonas*, *Enterococcus*, *Bacillus*, and *Staphylococcus* support digestion and survival under harsh conditions [8, 15], while *Pseudomonas* and *Proteus* contribute to nutrient metabolism and antibiotic resistance [7, 16]. *Pseudomonas* and *Bacillus* produce antimicrobial peptides (AMPs) such as blattellicin, which reduce pathogen load and enhance phenoloxidase activity [17]. *Blattabacterium cuenotii* synthesizes essential amino acids and vitamins and stimulates immunity [1, 18]. *Enterococcus faecalis* and *E. faecium* stimulate the production of defensins and bacteriocins that help eliminate multidrug-resistant (MDR) pathogens such as *Salmonella* sp. and *E. coli* [19]. *Lactobacillus* produces lactic acid and bacteriocins inhibit pathogens and modulate the Toll and Imd pathways [20, 21]. MDR pathogens are of growing concern because they resist most antibiotics and increase patient mortality, morbidity, and treatment costs [22]. Many studies indicate that the *B. germanica* gut microbiota carries genes encoding antimicrobial compounds that can inhibit MDR pathogens [23, 24]. Cockroach gut isolates, including *Serratia marcescens*, *E. coli*, *Klebsiella*, *Bacillus*, and *Streptococcus* [25], and *B. licheniformis*, *B. subtilis*, and *Kocuria rosea* from *Blattella vaga* [26], showed antibacterial activity against MDR pathogens. Other cockroach gut bacteria were active against pathogens such as *M. luteus*, MRSA, *S. pyogenes*, *B. cereus*, *E. coli* K1, *P. aeruginosa*, *K. pneumoniae*, *S. enterica*, *S. marcescens*, and *B. subtilis* [27, 28 and 29]. Cockroach gut bacteria are therefore a promising source of antibiotics or probiotics to help address the global antimicrobial resistance crisis [17, 28 and 30]. Accordingly, this study aimed to isolate bacteria from the foregut, midgut, and hindgut of adult *B. germanica* using five different culture media to recover a broad diversity of strains, characterize them by Gram staining and selected biochemical tests, and screen them for antagonistic activity against MDR pathogens.

Materials and Methods

2.1. Insect rearing and gut bacterial isolation

Adult of *B. germanica* were obtained from the Entomology Laboratory, Faculty of Science, Benha University, Egypt. A laboratory colony was maintained under controlled environmental conditions (27 ± 2°C and 65 ± 5% relative humidity). The wooden rearing cages were fitted with a glass top and covered with muslin cloth. Corrugated cardboard sheets were placed inside each cage to serve as harborage. The cockroaches were provided with white bread and water-soaked cotton pads [31, 32].

Before dissection, five adult cockroaches (weighing approximately 0.124 g each) were starved for 24 h. Each cockroach was individually sterilized three times with 70% ethanol for 2 s, followed by rinsing with sterile double-distilled water to remove the disinfectant. Using sterile scissors, a lateral incision was made behind the head capsule to isolate the gut. The gut was separated into foregut, midgut, and hindgut. Each gut section was homogenized in 1 mL of phosphate-buffered saline on a sterile porcelain plate and transferred into sterile Eppendorf tubes. Serial dilutions (10^{-1} to 10^{-6}) were prepared using sterile double-distilled water. Each dilution was plated on sterile Petri dishes containing nutrient agar (NA) media and incubated at 37 ± 1°C for 48 h. Bacterial load was assessed by counting colony-forming units (CFU/mL), and colony diameters were measured in centimeters (cm). The entire procedure was repeated three times. All dissections were performed under sterile conditions inside a biological safety cabinet [33].

2.2. Morphological and Biochemical Identification of Gut Bacteria:

For presumptive identification, 10 µL of each isolate was cultured on MacConkey (MAC), Eosin methylene blue (EMB), Mannitol Salt Agar (MSA), and Cetrimide (CE) agar using the spread plate method, then incubated at 37°C for 24 h. Bacterial species were initially characterized based on colony size, color, shape, margin, and elevation as described by [34].

The isolates were further characterized using Gram staining and selected biochemical tests according to the guidelines provided in Bergey's Manual of Systematic Bacteriology [35]. Additional tests, including methyl red and Voges–Proskauer reactions [36], indole production [37], and sugar fermentation [38, 39] were performed to further identify the isolated gut bacteria.

2.3. Antimicrobial activity assay:

2.3.1. Test Isolates:

The MDR microbial strains, including Gram-positive (GP) *S. aureus* **(**ATCC-23235**)** and *B.cereus* (ATCC- 14579), Gram-negative (GN) *E. coli* (ATCC-25922), *S. enteritidis* (ATCC-15566), as well as fungal strain, *C. albicans* (ATCC-10231), were obtained from the National Research Center, Cairo, Egypt.

An in vitro antimicrobial assay was conducted to evaluate the antibacterial and antifungal activities of gut bacteria isolated from the fore, mid, and hindgut of *B. germanica* adults against the previously mentioned clinical strains. The agar well diffusion method was employed for this purpose. Microbial inocula were prepared in nutrient broth for bacteria or distilled water for fungi, and suspensions were standardized to a concentration of 10^7 CFU/mL.

100 µL of MDR pathogen broth was evenly spread across the surface of NA plates for bacteria or potato dextrose agar (PDA) for fungal pathogens, using sterile cotton swabs, and wells of 6 mm diameter were then prepared in the agar using a sterile cork borer. 100 µL of the gut bacterial broth isolated from *B. germanica* was dispensed using a micropipette with sterile tips into each well. The plates were incubated under standard growth conditions (37 ± 1°C, for 24h). Antimicrobial activity was determined by measuring the diameter of the inhibition zones around each well in centimeters (cm), as described by [28]. Gut bacterial isolates that produced measured inhibition zones were selected for further molecular identification.

2.4. Molecular identification of gut bacteria

2.4.1. DNA extraction: A single colony of each isolate was separately inoculated in 10 mL of nutrient broth in Falcon tubes and incubated in a shaker overnight at 37°C, 100 rpm, then centrifuged at 12,000 rpm for 1 min to remove supernatant. The pellet DNA was extracted using a Genomic DNA Isolation Kit (Blood/Cultured Cell/Fungus) (GeneDireX, Inc., Taiwan, Cat. NA023-0100). The DNA was checked for quality according to the manufacturer's protocol by running it on 1% agarose gel, and then, its concentration was quantified using a Nano-Drop spectrophotometer [40].
2.4.2. PCR production: PCR was performed in a 20 µL reaction volume containing 12.5 µl of PCR master mix, 1 µM each of the forward primer 27 / reverse primers 1492, 2 µL of DNA template, and 3.5 µL nuclease-free water, then performed 40 amplification cycles at 95°C for 45 sec, 55°C for 30 sec, and 72°C for 90 sec.

2.4.3. Sequencing: Sequencing was performed at the Animal Health Research Institute (Al Dokki, Cairo, Egypt) where the purified PCR products were sequenced by using the general primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (3'-GGTTACCTTGTTACGACTT-5') [41]. Both these primers amplify the V3- V4 region of the 16S r RNA. Sequencing was performed by using BigDye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer, Foster City, CA) cat-number 4336817. Then centrisep (spin column), cat number CS-901 kit used to purify the sequence reaction. Sequencing products were resolved on an Applied Biosystem (ABI) model 3130 genetic analyzer (Hitachi, Japan) and nucleotides and amino acid sequences analyzed using Bioedit software, version 7.1, [42]. Sequence similarities were conducted using the BLASTn search at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and a phylogenetic and molecular evolutionary tree was conducted using MEGA 12.

2.4.4. Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using SPSS software. Means were separated for significance by Duncan's Multiple Range Test at $p \leq 0.05$. SE, standard errors, of triplicate measurements.

Results

3.1. Identification of gut bacteria isolated from adult of *B. germanica*

Seven bacterial isolates were isolated from the gut and coded as F1, F2, and F3 for bacteria isolated from the foregut, M1 and M2 for bacteria isolated from the midgut, while H1 and H2 are hindgut bacterial isolates. Table 1 reveals the morphological features of gut bacteria are isolated from the adult of *B. germanica*, all the bacterial colonies were creamy, except the H1 strain, which was yellow color. On MAC and EMB media, only F1 and F2 were white. F3, M1, M2, and H2 did not grow in both media. H1 had a white color on MAC media; however, it was colorless on EMB media. On MSA media, M2 and H2 produced pink, and F3 was yellow. Although F1, F2, M1, and H1 didn't grow on MSA media. On CE media, only F1 and F2 had a white color, but the remaining isolates didn't grow.

Table 1

Morphological features of the gut bacteria isolated from the adult of B. germanica

Gut bacterial codes						Shape	Gram staining	Count of bacteria*10^7 (CFU)/ml On the NA media (Av. ± SE)	Total Count of bacteria*10^7/ml On the NA media (Av. ± SE)	Colony diameter (cm) (Av. ± SE)
	NA	MAC	EMB	MSA	CE					
F1	Creamy	white	white	-	white	rod	-	10 ± 0.2^{b}	88 ± 0.2^{a}	0.2 ± 0^{a}
F2	Creamy	white	white	-	white	rod	-	$8 \pm 0.1b^{c}$		0.1 ± 0^{a}
F3	Creamy	-	-	Yellow	-	sphere	+	70 ± 0.3^{a}		0.06 ± 0^{a}
M1	Creamy	-	-	-	-	rod	+	8 ± 0.2^{bc}	31 ± 0.1^{b}	0.5 ± 0^{a}
M2	Creamy	-	-	pink	-	sphere	+	23 ± 0.1^{b}		0.2 ± 0^{a}
H1	Yellow	white	colorless	-	-	rod	-	3.67 ± 0.1^{c}	21 ± 0.15^{c}	0.23 ± 0.058^{a}
H2	Creamy	-	-	pink	-	sphere	+	17.33 ± 0.1^{b}		0.053 ± 0.0058^{a}

a, b & c: Mean having different letters within the same column are significantly different at $P \leq 0.05$ using one-way ANOVA.F1, F2, and F3 codes are the gut bacteria isolated from the foregut. M1 and M2 for bacterial isolates from the midgut, while H1 and H2 are hindgut bacterial isolates. NA: Nutrient, MAC: MacConkey, EMB: Eosin methylene blue, MSA: Mannitol, and CE: Cetrimide agar.

The total count of gut bacteria varied significantly and ranged from 21–88*10^7 CFU/ml on NA media. The foregut had the highest mean count (88x$10^{7)}$ CFU/ml followed by the midgut (31x10^7 CFU/ml), while the hindgut recorded the lowest value, 21 x10^7 CFU/ml. The diameter of the gut bacterial colony ranged from 0.06 to 0.5 cm, with no significant differences among them. According to Gram staining, the bacterial isolates were identified as bacilli (rods) and cocci (spheres). Three were GN bacilli, one was GP bacillus, and three were GP cocci.

Also, Table 2 shows the biochemical tests that confirmed the morphological results. All bacterial isolates were negative for the lactose fermentation test. F1 and F2 were positive for catalase, esterase, urease, sucrose fermentation, and aerobic tests. In contrast, isolates F3, M, and M2 were negative for indole and phosphatase tests. However, both H1 and H2 were positive for urease, lipase, and aerobic tests. Based on these characterizations, the isolated gut bacteria were classified into three phyla: Proteobacteria, Firmicutes, and Actinobacteria. Three bacterial isolates (F3, M1, and M2) belonged to Firmicutes, while F1, F2, and H1 bacterial codes belonged to Proteobacteria. Only H2 isolated from the hindgut belonged to Actinobacteria. Figure 1 shows that the percentage of each isolated bacterial strain was 50% (*S. sciur)*, 16.42% (*Paenibacillus* sp*)*, 12.38% (*M. luteus)*, 7.14% (*Pseudomonas* sp.*)*, 5.71% (*P. mohnii)*, 5.71% (*S. saprophyticus)*, and 2.63% (*Proteus sp.).*

Table .2. Biochemical identification of the gut bacteria isolated from the adult of *B. germanica*

Gut Bacterial codes	**Biochemical tests**										
	Vp Proskauer tests	**Indole test**	**Motility**	**Catalase**	**Oxidase**	**Esterase**	**Formation of spores**	**Fermentation of Glucose**	**Fermentation of Lactose**	**Fermentation of Sucrose**	**Aerobic**
F1	-	-	-	+	+	+	-	-	-	+	+
F2	-	-	-	+	+	+	-	-	-	+	+
F3	+	-	+	+	-	+	-	-	-	+	+
M1	-	-	+	+	-	+	+	-	-	+	+
M2	-	-	-	+	-	+	-	-	-	+	+
H1	+	+	+	+	-	+	-	+	-	-	+
H2	+	-	+	-	-	+	-	-	-	+	+

F1, F2, and F3 codes are the gut bacteria isolated from the foregut. M1 and M2 for bacterial isolates from the midgut, while H1 and H2 are hindgut bacterial isolates.

3.2. Antagonistic activity of gut bacteria

The antagonistic interactions between the gut bacteria isolated from *B. germanica* adults and MDR pathogens was performed using the agar-well diffusion method to evaluate the inhibition zone diameter (cm) after 24 h (Fig. 2). Among all tested isolates, the *Pseudomonas* sp. *strain AYA1* and *P. mohnii.* isolated from the foregut have significant antibacterial and antifungal properties against MDR pathogens. *Pseudomonas* sp. *strain AYA1* exhibits the strongest potential against all tested MDR pathogens, *C. albicans, E. coli*, *S. aureus, B. cereus*, and *S. enteritidis* (3.5, 2.6, 2.3, 1.9, and 1.1 cm, respectively). Followed by *P. mohnii*, which inhibited all MDR pathogens except *S. enteritidis* and *B. cereus*. In contrast, the remaining isolates showed no significant antagonistic activity against all tested MDR pathogens.

3.3. Molecular identification of antagonistic gut bacteria:

Further confirmation with 16S rRNA sequencing techniques was used to identify the two isolates that had antibacterial and antifungal activity against the tested MDR pathogens. Figure 3 shows a 1% agarose gel of DNA and PCR product, BLASTn alignment, and phylogenetic tree analysis of the assembled 16r RNA exhibited the highest similarities with previously partially sequenced 16S rRNA of *Pseudomonas Sp.*, and *P. mohnii.* on the NCBI website (Fig. 4). Both *Pseudomonas* sp. *strain AYA1* and *P. mohnii.* 16S ribosomal RNA gene were deposited in the Gene Bank database and were assigned to the accession numbers PV936963 and PZ298788, respectively (Table 3).

Table 3
GenBank accession details of gut bacteria of *B. germanica* adult identified by using 16S rRNA

Gut bacterial codes	Organism	GenBank accession number	Similarity (%)
F1	**Pseudomonas sp. strain AYA1**	**PV936963**	**100**
F2	**Pseudomonas mohnii**	**PZ298788**	**100**
F1, F2 codes are the gut bacteria isolated from the foregut			

Discussion

The rapid emergence and global dissemination of MDR pathogens represent one of the most pressing public health challenges, emphasizing the urgent need to discover novel antimicrobial agents. Natural products have attracted increasing interest as alternative sources of bioactive compounds, and insect-associated microbiota have recently emerged as promising reservoirs of antimicrobial metabolites owing to their adaptation to microbe-rich environments [7, 43 and 44]. In the present study, bacteria isolated from different gut parts of adult *B. germanica* belonged to the classes Gammaproteobacteria, Bacilli, and Actinomycetes, representing the phyla Proteobacteria, Firmicutes, and Actinobacteria. These findings agree with previous culture-dependent and molecular studies reporting these bacterial groups as dominant members of the core gut microbiota of *B. germanica* [9, 15 and 45]. In contrast, other investigations have identified Bacteroidota, Firmicutes, and Fusobacteria as the predominant gut-associated phyla [6, 46]. Such inconsistencies are likely attributable to differences in cockroach populations, environmental conditions, diet, and, most importantly, the use of culture-dependent versus culture-independent methodologies, as conventional culturing recovers only viable and cultivable microorganisms.

The foregut contained the highest abundance of bacteria, followed by the midgut and hindgut. Similar observations have been reported by Tinker and Ottesen [15], Tinker [47], and Jahnes [48] who attributed the high bacterial load in the foregut to its role as a temporary storage chamber where ingested food and associated microorganisms accumulate before digestion. Conversely, several sequencing-based studies have shown that the hindgut generally harbors the greatest bacterial density because of its anaerobic conditions and favorable environment for microbial fermentation [24, 49]. These contrasting findings likely reflect methodological differences as well as variation in environmental exposure, host diet, and cockroach populations.

Three bacterial isolates were recovered from the foregut, including *Pseudomonas* sp. *strain AYA*, *P. mohnii*, and *S. sciuri*, whereas the midgut yielded *S. saprophyticus* and *Paenibacillus* sp., and the hindgut contained *Proteus* sp. and *M. luteus*. The predominance of *Pseudomonas* and *Staphylococcus* species in the foregut is consistent with previous reports describing these genera as common members of the cockroach gut microbiota [8, 15]. Members of these genera have been implicated in nutrient metabolism, adaptation to environmental stress, and protection against invading microorganisms, thereby contributing to host fitness. Likewise, *Proteus* species have been associated with nutrient metabolism and microbial adaptation within insect gut ecosystems [16]. Nevertheless, the bacterial composition reported in different studies varies considerably. For example, Guzman and Vilcinskas [7] identified *Serratia* as a dominant foregut-associated genus, whereas Pan *et al.* [1] recovered only *Bacillus* species from the foregut. Similarly, Pérez-Cobas *et al.* [9] reported Firmicutes and Proteobacteria as dominant members of the midgut microbiota, while Dukes *et al.* [50] demonstrated that the hindgut is enriched with Bacteroidota, Firmicutes, Proteobacteria, and Desulfobacterota. Such variation highlights the dynamic nature of insect gut microbial communities, which are influenced by host diet, environmental conditions, geographic location, and methodological approaches [51].

A notable finding of the present study is the isolation of *P. mohnii* from the gut of *B. germanica*. To the best of our knowledge, this is the first report of this species from an animal host. Previous studies have recovered *P. mohnii* exclusively from engineered environmental habitats, particularly sequencing batch bioreactors treating paper mill wastewater [52]. This finding expands the known ecological distribution of *P. mohnii* and suggests that cockroach gut environments may harbor previously unrecognized bacterial taxa with potential ecological and biotechnological significance.

Among all bacterial isolates, the foregut-derived *Pseudomonas* sp. exhibited the strongest inhibitory activity against the tested MDR pathogens, indicating considerable potential for the production of antimicrobial metabolites. Followed by *P. mohnii* which inhibited all MDR pathogens except *S. enteritidis* and *B. cereus*. Although studies investigating the direct antibacterial activity of individual cockroach gut isolates against MDR human pathogens remain limited, previous research supports the antimicrobial potential of cockroach-associated bacteria. Zhang *et al.* [53] demonstrated that *P. reactans* isolated from the

gut of *B. germanica* inhibited the entomopathogenic fungus *Beauveria bassiana*, suggesting a role in microbial competition and host defense. Likewise, Zhang *et al.* [54] reported that gut-associated bacteria of *B. germanica* exhibited antifungal activity, with *Pseudomonas* among the dominant bacterial genera contributing to microbial antagonism.

Evidence from other cockroach species further supports the antimicrobial potential of insect-associated microbiota. Ali *et al.* [55] demonstrated that extracts from *P. americana* exhibited antibacterial activity against MRSA and neuropathogenic *Escherichia coli* K1. Similarly, Amer *et al.* [44] reported that gut bacterial isolates from *P. americana* inhibited several multidrug-resistant bacterial and fungal pathogens, whereas 28 Akbar *et al.* [28] showed that gut bacteria isolated from *Gromphadorhina portentosa* and *Blaptica dubia* produced antibacterial compounds active against clinically important pathogens, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Collectively, these findings support the concept that cockroach-associated microbiota might be represented as a valuable source of antimicrobial compounds.

Conclusion

This study demonstrated that the gut of adult *B. germanica* harbors a diverse community of culturable bacteria belonging to the phyla Proteobacteria, Firmicutes, and Actinobacteria, with distinct bacterial populations distributed among the foregut, midgut, and hindgut. Notably, the foregut-derived *Pseudomonas* sp. *strain AYA1* exhibited the strongest inhibitory activity against the tested MDR pathogens, highlighting its potential as a promising source of antimicrobial metabolites. Furthermore, this study reports, for the first time, the isolation of *P. mohnii* from an animal host, expanding the known ecological distribution of this species. Collectively, these findings indicate that the gut microbiota of *B. germanica* represents a valuable reservoir of bacteria with antimicrobial potential. Future studies should focus on the isolation, purification, and structural characterization of the bioactive compounds responsible for the observed antimicrobial activity, as well as elucidating their mechanisms of action and evaluating their therapeutic potential against MDR pathogens.

Declarations

Author Contribution

M.F.A., A.R.A., M.S.A., O.M.D., and H.F.A. conceived and designed the research. M.F.A., A.R.A., and H.F.A. performed insect rearing, dissections, bacterial isolation, and biochemical characterization. M.S.A. and O.M.D. carried out antimicrobial assays, molecular identification, 16S rRNA sequencing, and phylogenetic analyses. M.F.A., A.R.A., and M.S.A. performed statistical analysis and data visualization. O.M.D. and H.F.A. drafted and revised the manuscript text. All authors reviewed and approved the final manuscript.

Data Availability

The 16S rRNA gene sequence data generated in this study have been deposited in the NCBI GenBank database under accession numbers PV936963 (for Pseudomonas sp. strain AYA1) and PZ298788 (for Pseudomonas mohnii). All other datasets generated or analyzed during the study are included within the manuscript.

References

1. Pan, X., Wang, X. & Zhang, F. New insights into cockroach control: using functional diversity of *Blattella germanica* symbionts. *Insects* **11** (10), 696. https://doi.org/10.3390/insects11100696 (2020).
2. Banerjee, P., Sarkar, A., Ghosh, K. & Mazumdar, A. A metagenomic based approach on abundance and diversity of bacterial communities across the life stages of *Culicoides peregrinus* (Diptera: Ceratopogonidae) a vector of bluetongue virus. *J. Med. Entomol.* **60** (2), 373–383 (2023).
3. Jones, J. C. et al. The gut microbiome is associated with behavioural task in honey bees. *Insectes Soc.* **65** (3), 419–429. https://doi.org/10.1007/s00040-018-0624-9 (2018).
4. Gao, F. et al. Bacterial community composition in gut content and ambient sediment of two tropical wild sea cucumbers (*Holothuria atra* and *H. leucospilota*). *J. Oceanol. Limnol.* **40** (1), 360–372 (2022).
5. Vršanský, P. et al. Cockroaches probably cleaned up after dinosaurs. *PLoS One*. **8** (12). https://doi.org/10.1371/journal.pone.0080560 (2013). e80560.
6. Pietri, J. E., Tiffany, C. & Liang, D. Disruption of the microbiota affects physiological and evolutionary aspects of insecticide resistance in the German cockroach, an important urban pest. *PLoS One*. **13** (12). https://doi.org/10.1371/journal.pone.0207985 (2018). e0207985.
7. Guzman, J. & Vilcinskas, A. Bacteria associated with cockroaches: health risk or biotechnological opportunity? *Appl. Microbiol. Biotechnol.* **104** (24), 10369–10387. https://doi.org/10.1007/s00253-020-10973-6 (2020).
8. Menasria, T. et al. External bacterial flora and antimicrobial susceptibility patterns of Staphylococcus spp. and Pseudomonas spp. isolated from two household cockroaches, *Blattella germanica* and Blatta orientalis. *Biomed. Environ. Sci.* **28** (4), 316–320. 10.3967/bes2015.045 (2015).
9. Pérez-Cobas, A. E. et al. Diet shapes the gut microbiota of the omnivorous cockroach *Blattella germanica*. *FEMS Microbiol. Ecol.* **91** (4), fiv022. https://doi.org/10.1093/femsec/fiv022 (2015).
10. Rosas, T. et al. Rifampicin treatment of *Blattella germanica* evidences a fecal transmission route of their gut microbiota. *FEMS Microbiol. Ecol.* **94** (2), fiy002. https://doi.org/10.1093/femsec/fiy002 (2018).
11. Kakumanu, M. L., Maritz, J. M., Carlton, J. M. & Schal, C. Overlapping community compositions of gut and fecal microbiomes in lab-reared and field-collected German cockroaches. *Appl. Environ. Microbiol.* **84** (17), e01037–e01018. https://doi.org/10.1128/AEM.01037-18 (2018).

12. Chen, B. et al. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci. Rep.* **6** (1), 29505. https://doi.org/10.1038/srep29505 (2016).
13. Schmidt, K. & Engel, P. Mechanisms underlying gut microbiota–host interactions in insects. *J. Exp. Biol.* **224** (2), jeb207696. https://doi.org/10.1242/jeb.207696 (2021).
14. Zhang, X., Zhang, F. & Lu, X. Diversity and functional roles of the gut microbiota in lepidopteran insects. *Microorganisms* **10** (6), 1234. https://doi.org/10.3390/microorganisms10061234 (2022).
15. Tinker, K. A. & Ottesen, E. A. The core gut microbiome of the American cockroach, *Periplaneta americana*, is stable and resilient to dietary shifts. *Appl. Environ. Microbiol.* **82** (22), 6603–6610. https://doi.org/10.1128/AEM.01837-16 (2016).
16. Holt, J. R., de Oliveira, C., Medina, N., Malacrinò, R. F., Lindsey, A. R. & A. & Insect–microbe interactions and their influence on organisms and ecosystems. *Ecol. Evol.* **14** (7), e11699. https://doi.org/10.1002/ece3.11699 (2024).
17. Siddiqui, R., Elmashak, Y. & Khan, N. A. Cockroaches: a potential source of novel bioactive molecule (s) for the benefit of human health. *Appl. Entomol. Zool.* **58** (1), 1–11 (2023).
18. Domínguez-Santos, R. et al. Gut microbiota is not essential for survival and development in *Blattella germanica* but affects uric acid storage. *Life* **14** (1), 153. https://doi.org/10.3390/life14010153 (2024).
19. Sahoo, A. et al. Antimicrobial peptides derived from insects offer a novel therapeutic option to combat biofilm: A review. *Front. Microbiol.* **12**, 661195. https://doi.org/10.3389/fmicb.2021.661195 (2021).
20. Barad, B. D., Parmar, K. D., Nandaniya, M. G., Bhuva, K. J. & Bhatt, N. A. Guardians of the insect realm: symbiotic mediation for host protection. *Plant. Arch.* **25**, 217–226. 10.51470/PLANTARCHIVES.2025.SP.ICTPAIRS-034 (2025).
21. Kee, P. E. et al. Sustainable Solutions to Combat Antimicrobial Resistance in Healthcare and Food Systems: Insights and Way Forward. *Recent. Adv. Anti-Infect Drug Discov*. 10.2174/0127724344392092251121095547 (2026).
22. Delle Rose, D. et al. Microbiologic characteristics and predictors of mortality in bloodstream infections in intensive care unit patients: A 1-year, large, prospective surveillance study in 5 Italian hospitals. *Am. J. Infect. Control*. **43** (11), 1178–1183. 10.1016/j.ajic.2015.06.023 (2015).
23. Zawack, K., Love, W. J., Lanzas, C., Booth, J. G. & Gröhn, Y. T. Estimation of multidrug resistance variability in the National Antimicrobial Monitoring System. *Prev. Vet. Med.* **167**, 137–145. 10.1016/j.prevetmed.2019.03.006 (2019).
24. Domínguez-Santos, R. et al. Interkingdom gut microbiome and resistome of the cockroach *Blattella germani*a. *mSystems* 6(3), 10-1128 (2021). 10.1128/mSystems.01213-20
25. Malard, F., Dore, J., Gaugler, B. & Mohty, M. Introduction to host microbiome symbiosis in health and disease. *Mucosal Immunol.* **14** (3), 547–554. 10.1038/s41385-020-00365-4 (2021).
26. Alkhalifah, D. H. M. Evaluation of antimicrobial activity of bacterial symbionts isolated from wild field cockroach *Blattella vaga* from Saudi Arabia. *Saudi J. Biol. Sci.* **28** (11), 6239–6244. 10.1016/j.sjbs.2021.06.092 (2021).
27. Basseri, H. R., Dadi-Khoeni, A., Bakhtiari, R., Abolhassani, M. & Hajihosseini-Baghdadabadi, R. Isolation and purification of an antibacterial protein from immune induced haemolymph of American cockroach, *Periplaneta americana*. *J. Arthropod Borne Dis.* **10** (4), 519 (2016).
28. Akbar, N., Siddiqui, R., Iqbal, M., Sagathevan, K. & Khan, N. A. Gut bacteria of cockroaches are a potential source of antibacterial compound (s). *Lett. Appl. Microbiol.* **66** (5), 416–426. https://doi.org/10.1111/lam.12867 (2018).
29. Mahboub, M. T., Hassan, M. I., Bream, A. S., Mohamed, A. F. & Abdel-Samad, M. R. Evaluation of the antibacterial and antifungal activities of chitosan prepared from the American cockroach (*Periplaneta americana*). *Egypt. Acad. J. Biol. Sci. E Med. Entomol. Parasitol.* **13** (1), 39–46. 10.21608/eajbse.2021.186760 (2021).
30. Crespo, A. et al. Pathogens of medical importance identified in hospital-collected cockroaches: a systematic review. *Microorganisms* **13** (2), 337. https://doi.org/10.3390/microorganisms13020337 (2025).
31. El-Monairy, O. M., Ibrahim, A. W. A., Zedan, M. H. & Abdel-Meguid, A. D. Insecticidal activity of Silica, zinc, and Copper nanoparticles against the German cockroach, *Blatella germanica* (L.) (Dictyoptera: Blattellidae). *Egypt. Acad. J. Biol. Sci. Entomol.* **16** (1), 15–26. 10.21608/eajbsa.2023.282347 (2023).
32. El-Sayed, Y. A., Ibrahim, A. A. & Hegazy, M. Improving the efficiency of two local baits used for the control of the German cockroach, *Blattella germanica* (L.),(Dictyoptera: Blattellidae) (2017). 10.9790/3008-1205065966
33. Ragab, A., Abd-ElAziz, M. F., Esmael, A. & Baz, M. M. Gut microbial diversity and immunological effects of antibiotics on *Spodoptera littoralis* feeding on different diets. *Benha J. Appl. Sci.* **7** (12), 53–61. 10.21608/bjas.2022.306861 (2022).
34. Akter, A., Rahman, M. A. & Zerin, T. Isolation and Identification of Chromium-Tolerant Bacteria from Poultry Litter in Dhaka, Bangladesh. *Stamford J. Microbiol.* **14** (1), 18–21. https://doi.org/10.3329/sjm.v14i1.78627 (2024).
35. Dawwam, G. E., Daboura, E. M., Abdel-Monem, M. O., Al-Shemy, M. T. & Mansor, E. S. Bacteriological and Physicochemical Evaluation of Different Wells Water in El-Qalubia Governorate, Egypt. *Egypt. Acad. J. Biol. Sci. G Microbiol.* **15** (1), 179–189. 10.21608/eajbsg.2023.303301 (2023).
36. McDevitt, S. Methyl red and Voges-Proskauer test protocols. *Am. Soc. Microbiol.* **8**, 1–9 (2009).
37. MacWilliams, M. P. *Indole test protocol* 1–3 (American Society for Microbiology, 2012).
38. Cheesbrough, M. District laboratory practice in tropical countries, part 2. Cambridge University Press ISBN: 9780521676311 (2005).
39. Huang, S., Sheng, P. & Zhang, H. 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 (2012).
40. Graham, J. E. et al. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. *Invest. Ophthalmol. Vis. Sci.* **48** (12), 5616–5623. https://doi.org/10.1167/iovs.07-0588 (2007).

41. Songnaka, N., Nisoa, M., Atipairin, A., Wanganuttara, T. & Chinnawong, T. Enhanced antibacterial activity of *Brevibacillus* sp. SPR19 by atmospheric and room temperature plasma mutagenesis (ARTP). *Sci. Pharm.* **90** (2), 23. https://doi.org/10.3390/scipharm90020023 (2022).
42. Xia, R., Ren, Y., Guo, X. & Xu, H. Molecular diversity of class 2 integrons in antibiotic-resistant gram-negative bacteria found in wastewater environments in China. *Ecotoxicology* **22** (2), 402–414. 10.1007/s10646-012-1034-9 (2013).
43. Váradi, L. et al. Methods for the detection and identification of pathogenic bacteria: past, present, and future. *Chem. Soc. Rev.* **46** (16), 4818–4832. 10.1039/c6cs00693k (2017).
44. Amer, A. et al. Antagonistic activity of bacteria isolated from the *Periplaneta americana* L. gut against some multidrug-resistant human pathogens. *Antibiotics* **10** (3), 294. https://doi.org/10.3390/antibiotics10030294 (2021).
45. Tegtmeier, D. et al. Culture-independent and culture-dependent characterization of the black soldier fly gut microbiome reveals a large proportion of culturable bacteria with potential for industrial applications. *Microorganisms* **9** (8), 1642. https://doi.org/10.3390/microorganisms9081642 (2021).
46. Carrasco, P. et al. Succession of the gut microbiota in the cockroach *Blattella germanica*. *Int. Microbiol.* **17** (2), 99–109. 10.2436/20.1501.01.212 (2014).
47. Tinker, K. A. Exploring the ecology of complex microbial communities through the cockroach gut microbiome. PhD thesis, North Carolina State University (2018).
48. Jahnes, B. C. Host-Microbial Symbiosis Within the Digestive Tract of *Periplaneta americana*. Graduate program in microbiology, The Ohio State University (2020).
49. Jahnes, B. C., Poudel, K., Staats, A. M. & Sabree, Z. L. Microbial colonization promotes model cockroach gut tissue growth and development. *J Insect Physiol* **133**, 104274 (2021): 10.1016/j.jinsphys.2021.104274
50. Dukes, H. E., Tinker, K. A. & Ottesen, E. A. Disentangling hindgut metabolism in the American cockroach through single-cell genomics and Meta transcriptomics. *Front Microbiol* **14**, 1156809 (2023): https://doi.org/10.3389/fmicb.2023.1156809
51. Yao, Y. H. et al. Structure, diversity and function prediction of intestinal tract bacterial composition of *Paralipsa gularis* (Zeller), a new maize pest : (2024). 10.3969/j.issn.2095-1191.2024.02.006
52. Cámara, B. et al. *Pseudomonas reinekei* sp. nov., *Pseudomonas moorei* sp. nov. and *Pseudomonas mohnii* sp. nov., novel species capable of degrading chlorosalicylates or isopimaric acid. *Int. J. Syst. Evol. Microbiol.* **57** (5), 923–931. https://doi.org/10.1099/ijs.0.64703-0 (2007).
53. Zhang, F. et al. Pseudomonas reactans, a bacterial strain isolated from the intestinal flora of *Blattella germanica* with *anti-Beauveria bassiana activity*. *Environ. Entomol.* **42** (3), 453–459. https://doi.org/10.1603/EN12347 (2013).
54. Zhang, F. et al. The interactions between gut microbiota and entomopathogenic fungi: a potential approach for biological control of *Blattella germanica* (L). *Pest Manag Sci.* **74** (2), 438–447. https://doi.org/10.1002/ps.4726 (2018).
55. Ali, S. M. et al. Identification and characterization of antibacterial compound(s) of cockroaches (*Periplaneta americana*). *Appl. Microbiol. Biotechnol.* **101** (1), 253–286. https://doi.org/10.1007/s00253-016-7872-2 (2017).

Figures

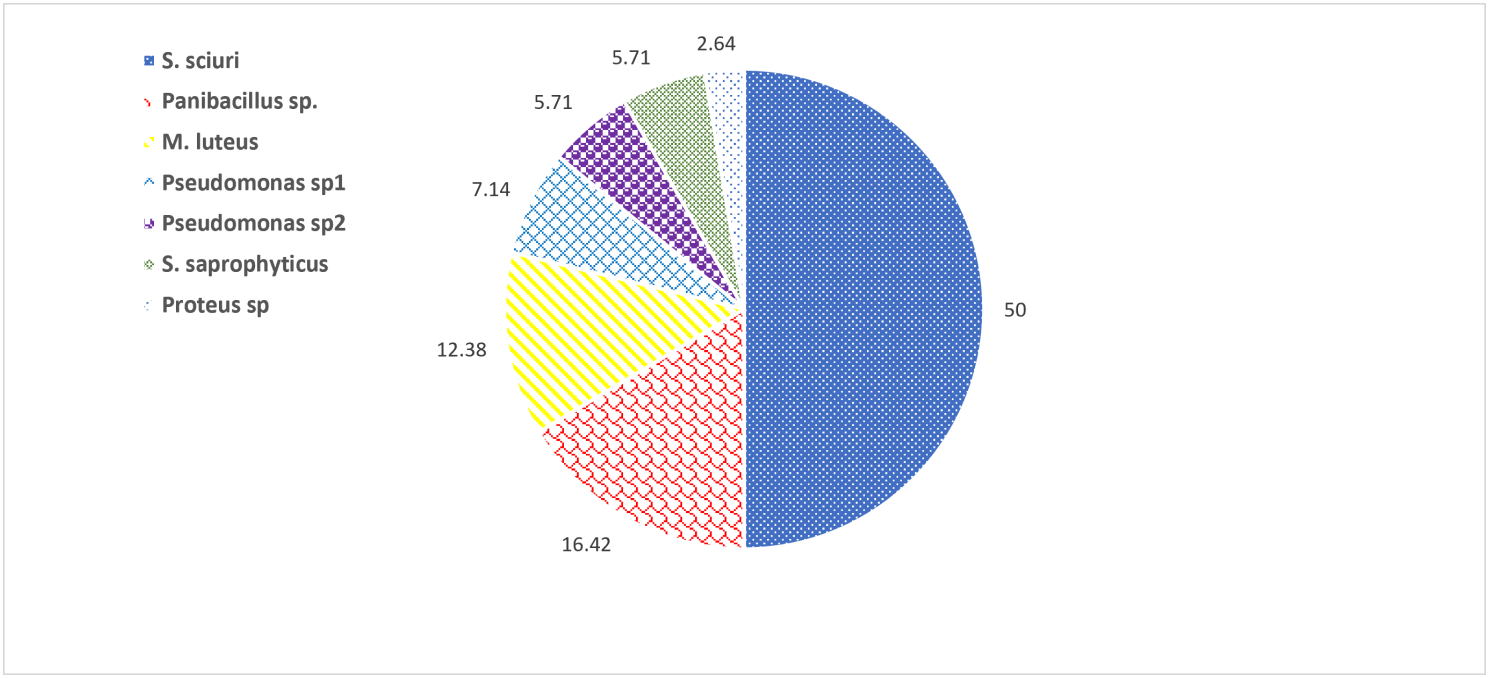

Figure 1

The frequency of occurrence (%) of gut bacteria isolated from the *B. germanica* adults.

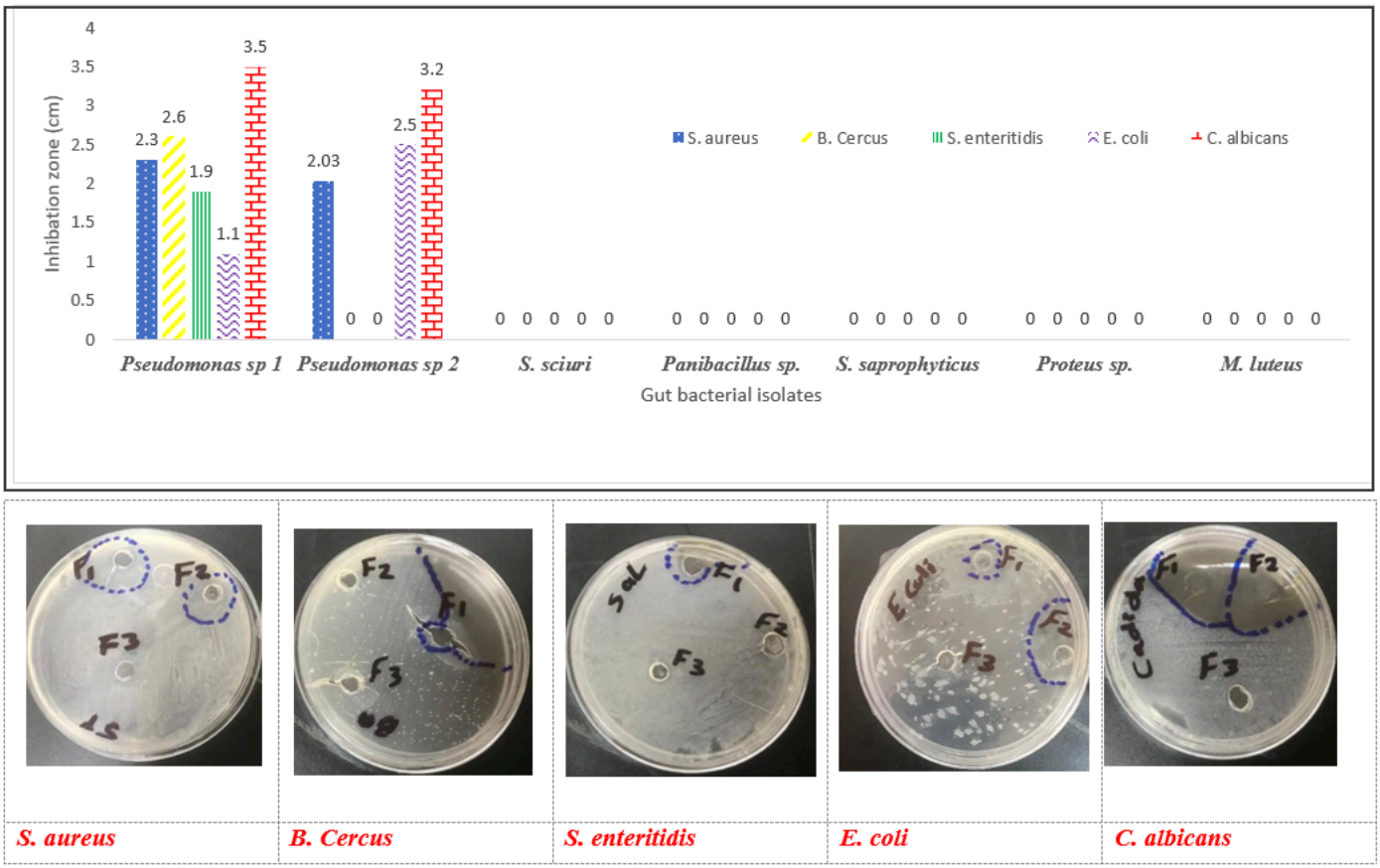

Figure 2

Antagonistic actisvity of bacterial gut isolates against tested MDR pathogens after 24. F1: *Pseudomonas* sp1; F2: *Pseudomonas* sp2, F3: s. *sciyri*; M1: *Paenibacillus* sp; M2: *S. saprophytics*; F1: *Proteus* sp; H2: *M. luteus*.

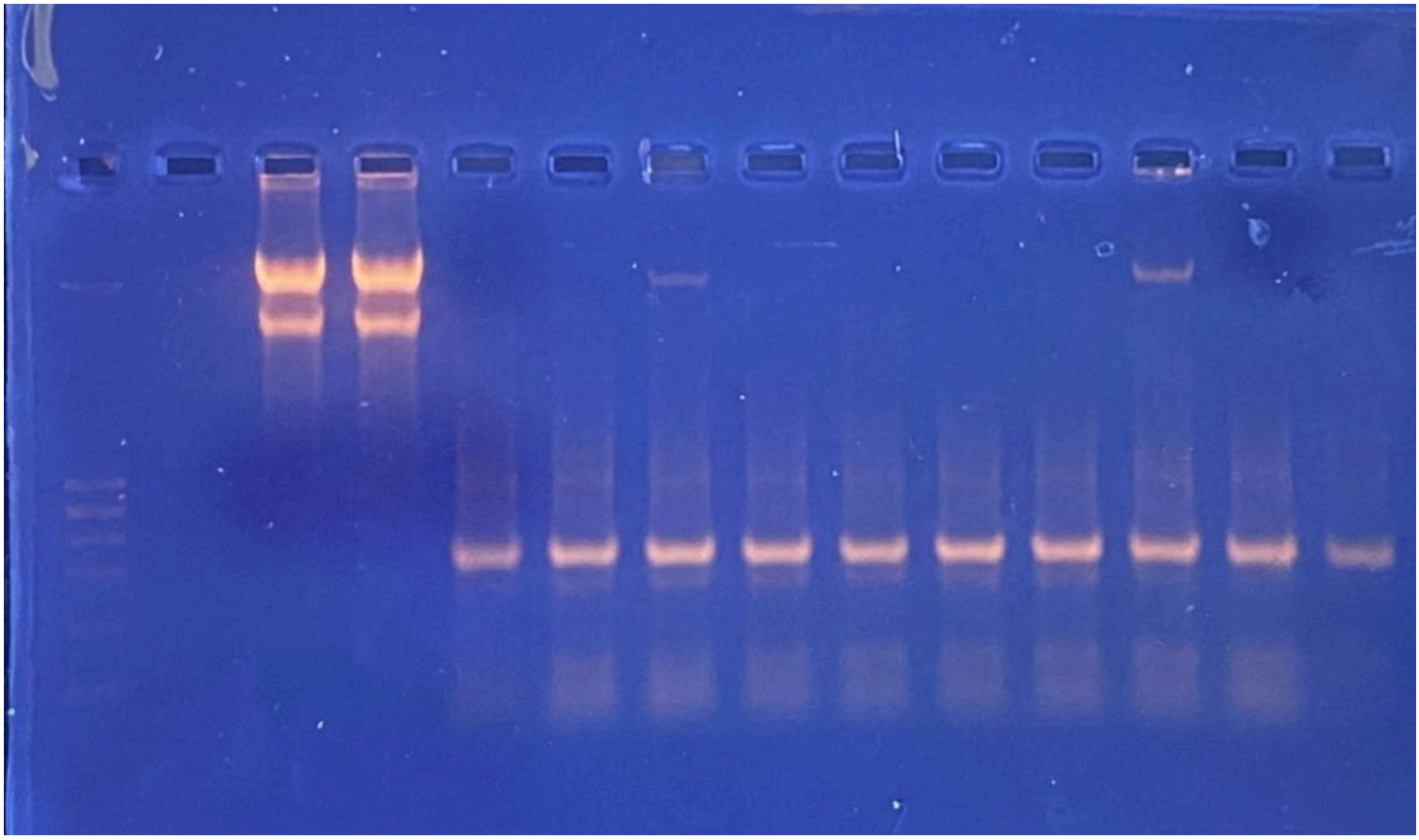

Figure 3

1% Agarose gel electrophoresis of genomic DNA and PCR product of gut bacteria isolated from the adult of *B. germanica*. 1: DNA ladder, 2: DNA of F1,3: DNA of F2, 4: PCR product of F1, 5: PCR product of F2.

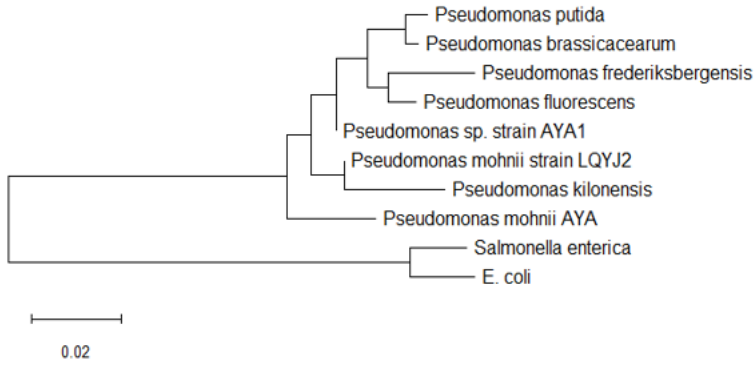

Figure 4

Phylogenetic tree shows the evolutionary relationships between the 16S rRNA sequences of gut bacterial isolated from *B. germanica* adult with concatenated nucleotide sequences of their 16s rRNA.