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Diversity and cellulolytic potential of gut bacteria isolated from *Blaberus* and *Panesthia* cockroaches in Aceh, Indonesia

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Abstract. *Sabariah S, Suhartono S, Rizki A. 2025. Diversity and cellulolytic potential of gut bacteria isolated from* Blaberus *and* Panesthia *cockroaches in Aceh, Indonesia. Biodiversitas 26: 5145-5155.* Forest-dwelling cockroaches harbor diverse gut microbiota capable of degrading complex organic compounds, particularly cellulose. This study investigated the diversity and cellulolytic potential of gut bacteria isolated from two cockroach genera, namely *Blaberus* and *Panesthia*, collected from the Soraya Research Station, Aceh, Indonesia. Ten individuals of each species were collected between January and March 2025 using standardized pitfall traps and hand collection methods. Through systematic isolation and characterization, a total of 19 bacterial isolates were isolated comprising 10 from *Blaberus* and 9 from *Panesthia*. Morphological and biochemical analysis revealed diverse bacterial genera, with *Blaberus* harboring *Klebsiella, Bacillus, Flavobacterium, Pseudomonas, Salmonella*, and *Staphylococcus*, while *Panesthia* contained predominantly *Bacillus* (55.5%) along with *Salmonella, Staphylococcus,* and *Enterococcus.* Three isolates from *Blaberus* demonstrated cellulose-degrading capabilities, *Bacillus, Flavobacterium*, and *Pseudomonas* with cellulolytic indices of 0.08, 2.45, and 3.08, respectively. Gas Chromatography-Mass Spectrometry analysis revealed high concentrations of linoleic acid in these cellulolytic isolates. These findings are the first study of cellulolytic bacteria from forest cockroaches in Indonesia's unique Aceh ecosystem, enhancing understanding of tropical forest nutrient cycling and demonstrating significant potential for sustainable biotechnological applications in enzyme production and bioconversion processes.

Keywords: *Blaberus*, cellulase activity, gut microbiota, insect symbionts, *Panesthia*

INTRODUCTION

Cellulose is the most abundant organic compound on Earth, consisting of unbranched chains of β-D-glucose-pyranose units linked by β-1,4-glycosidic bonds. Its structural complexity makes cellulose resistant to degradation, yet its hydrolysis into glucose offers vast potential for industrial and environmental applications. Microorganisms capable of secreting lignocellulolytic enzymes play a central role in this process, enabling environmentally sustainable solutions for biomass utilization (Ali et al. 2021; Turner et al. 2022). Among them, cellulolytic bacteria are particularly important as producers of cellulase enzymes, which are widely applied in biorefineries, agricultural residue processing, textile and detergent industries, as well as biofuel production (Ejaz et al. 2021; Seddiqi et al. 2021). The discovery of novel cellulolytic microorganisms adapted to tropical environments is therefore a strategic priority for advancing sustainable biotechnology.

Aceh Province, Indonesia, is a unique biogeographical region where the Indo-Malayan and Australasian faunal zones converge, resulting in exceptionally high biodiversity and endemism. The Soraya Research Station in Subulussalam represents a relatively undisturbed tropical rainforest ecosystem, providing an ideal setting to explore symbiotic interactions between insects and their microbial partners. Forest cockroaches, especially members of the family Blaberidae, play an important ecological role as detritivores, contributing to organic matter decomposition and nutrient cycling (Beza-Beza et al. 2024). Despite their ecological significance, the gut microbiota of forest cockroaches has received limited scientific attention compared with termites and beetles, which are more extensively studied for their lignocellulose-degrading microbiomes (Hedjouli et al. 2021; Bautista-Cruz et al. 2024).

Cockroaches are particularly interesting because of their detritivorous feeding habits, which include consumption of leaf litter, decaying wood, and other cellulose-rich substrates. Their gut microbiota provides essential digestive capabilities that the host alone cannot achieve, including enzymatic breakdown of complex polysaccharides (Jing et al. 2020; Osaki et al. 2024). Previous studies on omnivorous cockroaches have revealed phylosymbiotic patterns, where host diet and ecological niches strongly shape microbial community assembly (Tinker and Ottesen 2020; Renelies-Hamilton et al. 2021). However, very few studies have specifically addressed the diversity and cellulolytic potential of gut bacteria in forest cockroaches, particularly in Southeast Asia, despite the likelihood that these insects harbour novel microbial symbionts with valuable enzymatic capabilities (Li et al. 2023; Schapheer et al. 2024).

Among forest cockroaches, the genera *Blaberus* and *Panesthia* are of special interest because of their contrasting ecological strategies. *Blaberus* species are generalist feeders inhabiting a wide range of microhabitats, whereas *Panesthia* are primarily wood-feeding specialists (Schwarz

et al. 2023). Such differences in feeding ecology are expected to influence their gut microbial composition and functional traits. Studies in related insects have shown that microbial communities often determine host nutritional ecology, with *Bacillus* and *Pseudomonas* frequently identified as key contributors to cellulolytic processes (Bautista-Cruz et al. 2024). Furthermore, wood-feeding insects are known to rely on consortia of gut symbionts, which suggests that the discovery of cellulolytic bacteria from cockroaches may reveal both novel taxa and unique metabolic pathways (Jing et al. 2020; Turner et al. 2022).

In addition to enzymatic activity, microbial metabolites generated during cellulose degradation are increasingly recognized as having biotechnological importance. Gas Chromatography-Mass Spectrometry (GC-MS) analysis has identified compounds, such as linoleic acid and other fatty acids as byproducts of microbial activity, which may contribute to industrial applications ranging from biofuels to pharmaceuticals (Mirmohammadsadegh et al. 2021; Seddiqi et al. 2021). Investigating both cellulolytic enzyme activity and associated metabolite production is therefore crucial for evaluating the full biopotential of insect gut symbionts.

Despite the growing recognition of insect-associated microbiota as valuable sources of biocatalysts, there remains a significant gap in the study of forest cockroach symbionts, especially in Indonesia. Research to date has focused largely on termites and beetles, leaving cockroaches underexplored despite their ecological relevance and potential for harbouring novel cellulolytic bacteria. Addressing this gap is particularly important in tropical regions such as Aceh, where the unique environmental conditions may support microbial taxa with specialized enzymatic and metabolic capacities (Fitri et al. 2021; Bautista-Cruz et al. 2024).

The present study aimed to isolate, characterize, and evaluate the cellulolytic activity of bacteria obtained from the gut of two forest cockroach genera, *Blaberus* and *Panesthia*, collected from Aceh, Indonesia. In addition, the metabolic products of cellulolytic isolates were also analyzed using GC-MS to assess their potential biotechnological applications.

MATERIALS AND METHODS

Study area and sample collection

Cockroach specimens were collected from the Soraya Research Station, Subulussalam, Aceh, Indonesia (2°5'17.4" - 2°5'38.1" N, 97°55'41.5" - 97°55'51.2" E) between January and March 2025 (Figure 1). Collection sites represented diverse microhabitats including leaf litter and decaying wood areas within primary tropical rainforest. Ten individuals of each of *Blaberus* and *Panesthia* genera were collected to detect differences in bacterial diversity and cellulolytic activity. The combination of pitfall traps (for ground-dwelling species) and direct hand collection (for arboreal species) was employed to ensure representative sampling across different microhabitats and behavioral patterns. Live specimens were collected using a combination of pitfall traps and direct hand collection following standardized entomological protocols (Turner et al. 2022) and transported in sterile containers to the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, for immediate processing.

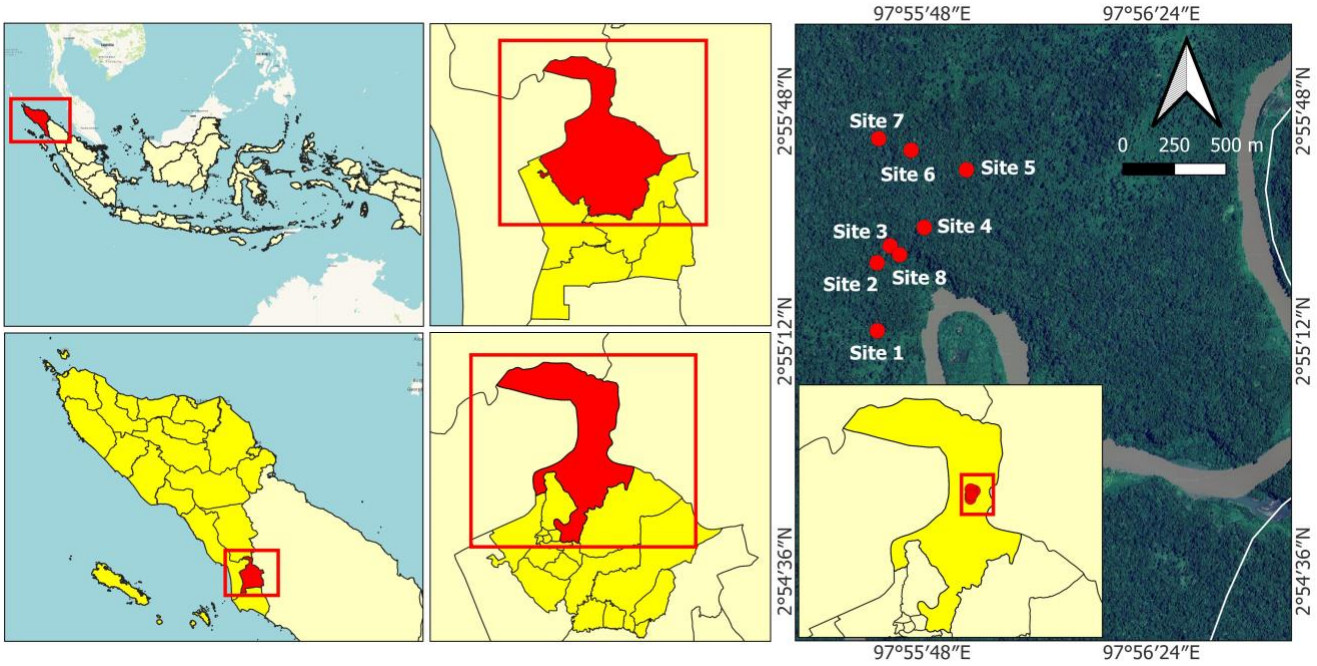


Figure 1. Sampling locations of forest cockroaches in Soraya Research Station, Aceh, Indonesia. Site 1: 2°5’17.4” N, 97°55’41.5” E, Site 2: 2°5’23.2” N, 97°55’39.8” E, Site 3: 2°5’26.1” N, 97°55’43.1” E, Site 4: 2°5’29.8” N, 97°55’47.7” E, Site 5: 2°5’35.3” N, 97°55’51.2” E, Site 6: 2°5’37.7” N, 97°55’46.8” E; Site 7: 2°5’38.1” N, 97°55’43.9” E; and Site 8: 2°5’25.0” N, 97°55’44.1” E

Figure 2. Specimens of forest cockroaches of: A. *Blaberus*, and B. *Panesthia*

Isolation and characterization of bacteria

Prior to bacterial isolation, *Blaberus* and *Panesthia* coackroaches (Figure 2) underwent a three-step surface sterilization protocol modified from Yulianti et al. (2023). The process involved an initial rinse in sterile 0.9% NaCl solution (2 min), followed by surface decontamination in 90% ethanol for 5 minutes, and a final rinse in sterile 0.9% NaCl solution (2 min). Sterilization efficacy was confirmed by plating the final rinse water on sterile agar plates. Specimens were then euthanized using chloroform vapor following ethical guidelines for insect research.

Under sterile conditions, the entire digestive tract was removed using flame-sterilized dissecting tools and homogenized in 10 mL sterile 0.9% NaCl solution. Serial dilutions (10^{-1} to 10^{-5}) were prepared for bacterial isolation using spread plate technique on Nutrient Agar and Tryptic Soy Agar media. Plates were incubated at 37°C for 24-48 hours. Morphologically distinct colonies were selected and purified through repeated streaking.

Characterization of bacterial isolates followed standard microbiological methods according to Bergey's Manual of Systematic Bacteriology. Macroscopic analysis examined colony morphology, including shape, size, margin, elevation, pigmentation, surface texture, and optical properties. Microscopic characterization involved Gram staining and observation of cell morphology, arrangement pattern, and spore formation when present. Biochemical characterization, included KOH test, catalase activity, MR-VP reaction, indole production, motility assessment, carbohydrate fermentation, H_2S production, and citrate utilization. Bacterial identification was based on morphological and biochemical characteristics using Bergey's Manual as reference.

Biopotency assay for cellulolytic activity

The cellulolytic index was defined as the ratio of clear zone diameter to colony diameter, with categories as weak (≤1.0), moderate (1.0-2.0), and strong (≥2.0). The cellulolytic activity test was conducted using Carboxy-Methyl Cellulose (CMC) agar medium containing CMC 10 g/L, agar 15 g/L, $MgSO_4 \cdot 7H_2O$ 0.02 g/L, $FeSO_4 \cdot 7H_2O$ 0.02 g/L, KNO_3 0.01 g/L, $CaCl_2 \cdot 2H_2O$ 0.01 g/L, dextrose 1 g/L, and yeast extract 1 g/L, pH 7.0. Pure isolates were point-inoculated in duplicates on CMC agar plates and incubated at 30°C for 7 days.

After incubation, plates were flooded with 0.1% Congo red solution for 15 minutes, followed by destaining with 1 M NaCl solution for 15 minutes (Golwalkar and Khatri 2023). Bacterial isolates capable of decomposing CMC were characterized by the formation of clear zones around colonies after Congo red treatment (Truong and Nguyen 2025). The cellulolytic activity index was calculated as the ratio of clear zone diameter to colony diameter, measured using digital callipers with two replicates per isolate.

$$\text{Cellulolytic Index} = \frac{[\text{Clear zone diameter (mm)-Colony diameter (mm)}]}{\text{Colony diameter (mm)}}$$

Crude extract cellulase enzyme production and Gas Chromatography-Mass Spectrometry (GC-MS) analysis

For metabolite analysis, cellulolytic isolates were cultured in liquid CMC medium (100 mL) at 30°C with shaking (120 rpm) for 24 hours. The cultures were centrifuged at 6000 rpm for 10 minutes, and the supernatants were filtered through Whatman No. 1 filter paper. GC-MS analysis was performed using a Shimadzu QP2010 Ultra system equipped with an RTX-5MS column (30 m × 0.25 mm × 0.25 µm). Helium served as the carrier gas at a flow rate of 1.0 mL/min. The injection temperature was set at 250°C, with an interface temperature of 280°C and ion source temperature of 200°C. The oven temperature program started at 60°C (2 min hold), increased at 10°C/min to 280°C, and held for 5 minutes. The injection volume was 1 µL with a split ratio of 1:10. Compound identification was performed by comparing mass spectra with the NIST library database (version 2020).

Data analysis

All experiments were conducted in duplicates, with data presented as mean±standard deviation. Statistical significance was determined using One-Way ANOVA with Tukey's post-hoc test ($p<0.05$) using GraphPad Prism 9.0 software.

RESULTS AND DISCUSSION

Bacterial isolation and characterization

A total of 19 bacterial isolates were successfully obtained from the gut contents of forest-dwelling cockroaches, comprising 10 isolates from *Blaberus* and 9 isolates from *Panesthia* specimens. The diversity observed refers to morphological and biochemical variation among isolates, rather than calculated diversity indices such as Shannon or Simpson indices. From *Blaberus* specimens, ten bacterial

strains (coded as BKB) were isolated, comprising three Gram-positive and seven Gram-negative bacteria. *Panesthia* specimens yielded nine isolates (coded as BKP), including four Gram-positive and five Gram-negative bacteria.

Morphological characterization revealed diverse colony characteristics among the isolates (Table 1). Bacteria isolated from *Blaberus* specimens predominantly exhibited round colonies with smooth margins and raised elevations, though some displayed distinctive features, such as concentric patterns (BKB-A1) and radiating margins (BKB-A4). Colony diameter ranged from 0.5 to 2.7 mm in diameter, with BKB-A3 showing the largest colony diameter (2.7 mm). Two isolates (BKB-B1 and BKB-C1) demonstrated irregular spreading growth patterns (Figure 3). *Panesthia* isolates showed similarly diverse morphological characteristics, with colony sizes ranging from 0.1 to 2.2 mm. Notably, isolate BKP-A3 produced distinctive purple pigmentation after 24 hours of incubation (Figure 4).

Biochemical characterization and subsequent genus-level identification using Bergey's Manual revealed distinct bacterial compositions between the two cockroach genera (Table 2). The gut microbiota of *Blaberus* comprised *Klebsiella* (20%), *Bacillus* (20%), *Flavobacterium* (20%), *Pseudomonas* (20%), *Salmonella* (10%), and *Staphylococcus* (10%). In contrast, *Panesthia*-derived isolates showed a different distribution pattern, with *Bacillus* as the dominant genus (55.5%), followed by equal proportions (11.1% each) of *Salmonella, Staphylococcus,* and *Enterococcus*. These differences in bacterial composition might suggest host-specific microbial associations.

Cellulose degradation activity test

Cellulolytic activity screening on CMC agar revealed that only three isolates from *Blaberus* specimens demonstrated cellulose-degrading capabilities (Table 3 and Figure 5). These included BKB-B1 (identified as *Bacillus*), BKB-C1 (*Flavobacterium*), and BKB-C2 (*Pseudomonas*). The cellulolytic indices varied among these isolates with *Pseudomonas* showing the highest activity (3.08±1.83), followed by *Flavobacterium* (2.45±0.54), while *Bacillus* exhibited relatively weak activity (0.08±0.06). Notably, none of the *Panesthia*-derived isolates showed detectable cellulolytic activity under the tested conditions (Figure 5).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Results of GC-MS analysis of the cellulolytic isolates revealed complex metabolic profiles, with 9,12-octadecadienoic acid (Z,Z)- emerging as the predominant compound in all three active strains. The concentration of this compound varied among the isolates with *Bacillus* showing the highest content (67.98%), followed by *Flavobacterium* (56.96%), and *Pseudomonas* (56.05%). Additional compounds identified included various fatty acids, alcohols, and esters, with each strain showing distinctive metabolic signatures. The *Bacillus* isolate produced 13 different compounds, while *Flavobacterium* and *Pseudomonas* yielded 16 and 12 compounds, respectively (Table 4).

Table 1. Macroscopic and microscopic characteristics of bacterial isolates from giant cockroaches (Blaberidae) at the Soraya Research Station, Subulussalam, Aceh, Indonesia

Habitats	Isolate codes	Colony characteristics			Cell characteristics			Pigmentation*
		Shapes	Size (mm)	Margins	Elevation	Shapes	Gram	
Litter (*Blaberus*)	BKB-A1	Concentric	1.8	Undulate	Raised	Bacilli	-	-
	BKB-A2	Round	0.5	Entire	Raised	Coccus	-	-
	BKB-A3	Round	2.7	Entire	Raised	Bacilli	-	-
	BKB-A4	Round with radating margin	1.1	Ciliate	Raised	Coccus	-	-
	BKB-A5	Round	0.8	Entire	Drop-like	Coccobacilli	+	-
	BKB-B1	Irregular and spreeding	n.a	Undulate	Raised	Bacilli	+	-
	BKB-B2	Round	0.8	Entire	Drop-like	Bacilli	-	-
	BKB-C1	Irregular and spreeding	n.a	Undulate	Flat	Bacilli	-	-
	BKB-C2	Round	0.8	Entire	Raised	Short Bacilli	-	-
	BKB-C3	Round	1.8	Entire	Raised	Coccus	+	-
Wood (*Panesthia*)	BKP-A1	Filamentous	1.5	Wooly	Raised	Bacilli	+	-
	BKP-A2	Round	0.9	Entire	Raised	Coccus	-	-
	BKP-A3	Round	0.1	Entire	Raised	Coccus	-	Produces purple pigment after 24 h
	BKP-B1	Round	1.2	Entire	Raised	Bacilli	-	-
	BKP-B2	Round with scaloped margin	1.5	Undulate	Raised	Bacilli	+	-
	BKP-B3	Round	0.6	Entire	Raised	Bacilli	-	-
	BKP-B4	Round with scaloped margin	0.8	Undulate	Flat	Bacilli	+	-
	BKP-C1	Round	0.1	Entire	Raised	Coccus	+	-
	BKP-C2	Round	2.2	Entire	Raised	Diplococcus	-	-

Note: *: Most isolates showed no distinctive pigmentation indicated by (-), except BKP-A3 which produced characteristic purple pigmentation

Table 2. Physiological characteristics of cellulose-degrading bacterial isolates from giant cockroaches (Blaberidae) at the Soraya Research Station, Subulussalam, Aceh, Indonesia

Isolate codes	KOH	Catalase	MR	VP	Indole	Motility	Fermentation of carbohydrate	H_2S	Citrate	Genus
BKB-A1	-	-	-	-	-	-	k/k	-	+	*Klebsiella*
BKB-A2	-	-	+	-	-	-	m/m	-	+	*Bacillus*
BKB-A3	-	-	+	-	+	-	k/k	-	+	*Salmonella*
BKB-A4	-	-	+	-	-	-	m/m	-	+	*Flavobacterium*
BKB-A5	+	-	-	-	-	-	m/k	-	+	*Staphylococcus*
BKB-B1	+	+	-	-	-	-	m/k	-	-	*Bacillus*
BKB-B2	-	-	+	+	-	-	k/k	-	+	*Klebsiella*
BKB-C1	-	+	-	-	-	-	m/k	-	+	*Flavobacterium*
BKB-C2	-	-	+	-	-	-	k/k	-	-	*Pseudomonas*
BKB-C3	+	-	+	-	-	-	k/k	-	-	*Pseudomonas*
BKP-A1	+	-	+	-	-	-	m/k	-	-	*Bacillus*
BKP-A2	-	-	+	-	-	-	k/k	-	+	*Bacillus*
BKP-A3	-	-	-	-	-	-	m/m	-	+	*Bacillus*
BKP-B2	+	-	+	-	-	-	m/k	-	+	*Bacillus*
BKP-B3	-	-	+	-	-	-	m/k	-	+	*Salmonella*
BKP-B4	+	-	-	-	-	-	k/k	-	-	*Bacillus*
BKP-C1	+	-	+	-	-	-	k/k	-	-	*Staphylococcus*
BKP-C2	-	-	+	-	-	+	m/k	+	+	*Enterococcus*

Note: +: Positive, -: Negative, m/k: Red butt yellow slant, k/k: Yellow butt yellow slant

Table 4. GC-MS analysis results of crude extract cellulase from selected bacterial isolates from giant cockroaches (Blaberidae) at the Soraya Research Station, Subulussalam, Aceh, Indonesia

Bacteria	Peaks	Compound names*	Retention time (min)	Areas* (%)	Simililarity index	Compound class
Bacillus	5	9,12-Octadecadienoic acid (Z,Z)-	23.048	67.98	93	Linoleic acid
	8	cis-9- Hexadecenal	25.769	15.63	87	Fatty aldehydes
Flavobacterium	6	9,12-Octadecadienoic acid (Z,Z)-	23.054	56.96	92	Linoleic acid
	9	cis-9- Hexadecenal	25.771	17.68	87	Fatty aldehydes
Pseudomonas	3	9,12-Octadecadienoic acid (Z,Z)-	23.054	56.05	92	Linoleic acid
	6	cis-9- Hexadecenal	25.772	18.10	87	Fatty aldehydes
	12	6-Nitro-cylohexadecane-1,3-dione	32.723	5.00	77	Dion nitro

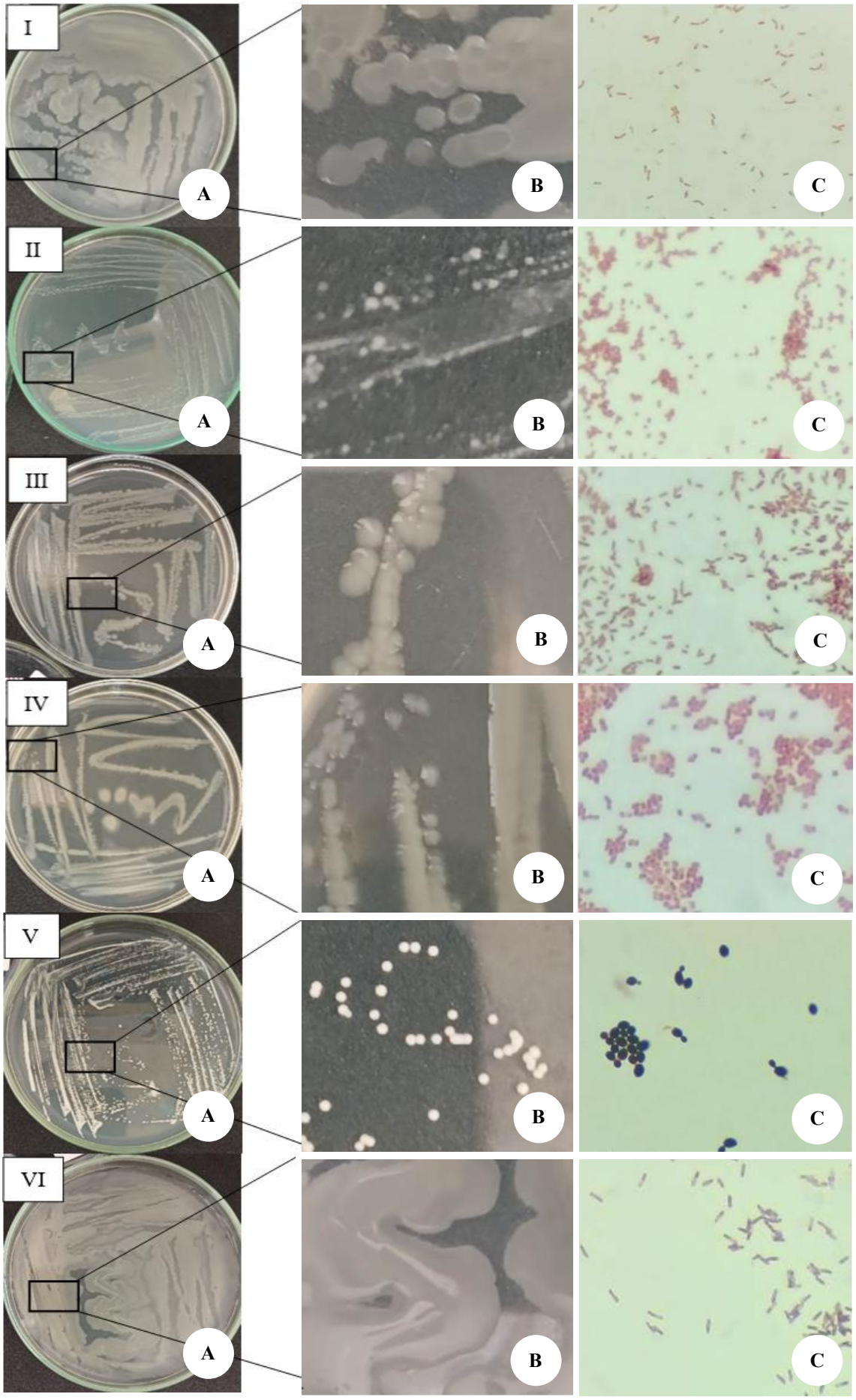
Note: *: Only major compounds (>5% abundance) shown for clarity

Table 3. Cellulose degradation activity of bacteria isolated from giant cockroaches (Blaberidae) at the Soraya Research Station, Subulussalam, Aceh, Indonesia

Isolate codes	Bacteria	Cellulolytic indices (mean±SD)	Activity level
BKB-B1	*Bacillus*	0.08 ± 0.06	Weak
BKB-C1	*Flavobacterium*	2.45 ± 0.54	Strong
BKB-C2	*Pseudomonas*	3.08 ± 1.83	Strong

Discussion

The diversity and functional capabilities of gut bacteria isolated from forest-dwelling cockroaches reveal intriguing patterns of host-microbe associations. The findings of the present study demonstrate distinct bacterial compositions between *Blaberus* and *Panesthia* genera, likely reflecting their different ecological niches and feeding behaviors. A more diverse bacterial community was observed in *Blaberus* (comprising *Klebsiella, Bacillus, Flavobacterium, Pseudomonas, Salmonella,* and *Staphylococcus*) compared to the *Bacillus*-dominated microbiota in *Panesthia*, similar results have been found in recent studies showing that insect gut bacterial communities are shaped by host diet and habitat (Tinker and Ottesen 2020; Renelies-Hamilton et al. 2021; Turner et al. 2022). The predominance of *Bacillus* in *Panesthia* (55.5%) suggests a special relationship with this bacterial genus, which is known for its ability to form endospores and survive harsh environmental conditions. This may be particularly advantageous for wood-feeding cockroaches that encounter variable nutrient availability and potentially toxic compounds in their diet. In contrast, the more balanced distribution of bacterial genera in *Blaberus* may reflect their more generalist feeding behavior and diverse microhabitat utilization.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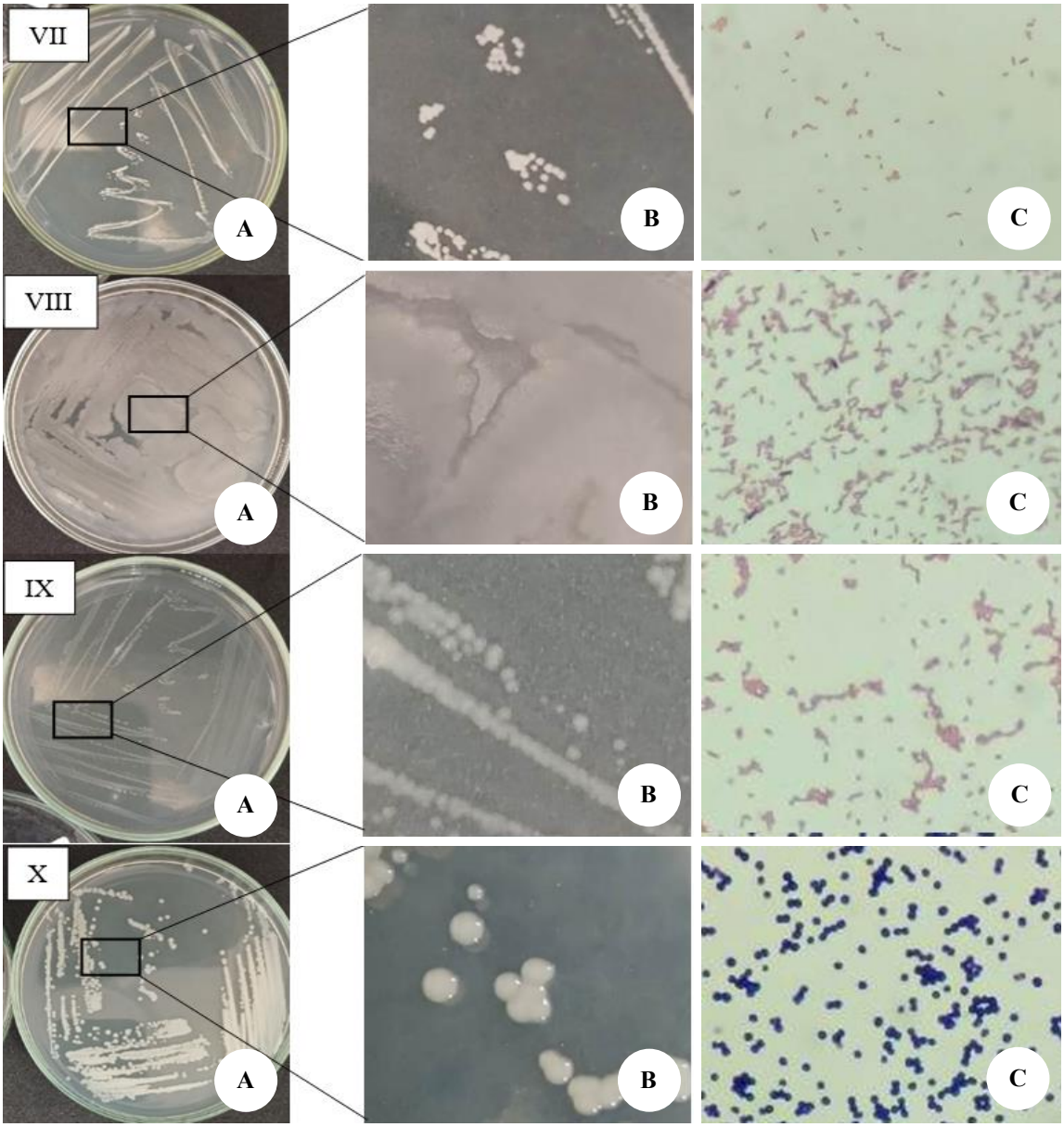
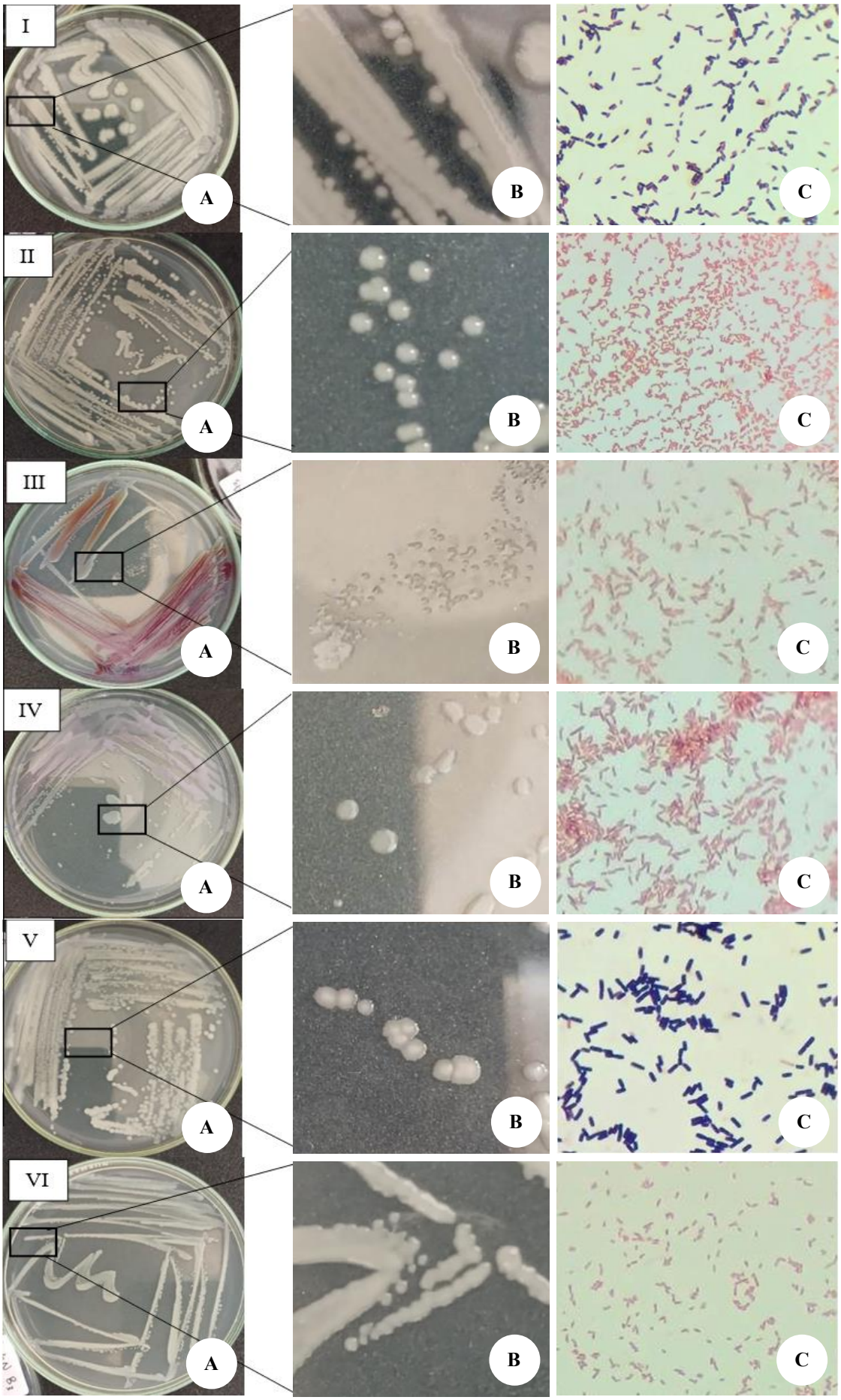


Figure 3. Bacterial morphology of isolates *Blaberus* at the Soraya Research Station, Subulussalam, Aceh, Indonesia. I. BKB-A1, II. BKB-A2, III. BKB-A3, IV. BKB-A4, V. BKB-A5, VI. BKB-B1, VII. BKB-B2, VIII. BKB-C1, IX. BKB-C2, X. BKB-C3. A. Colony morphological observation with their B. Magnification, and C. Gram-staining observed under microscope with 1000× magnification

The exclusive presence of cellulolytic activity in *Blaberus*-derived isolates, despite *Panesthia*'s wood-feeding habits, raises questions about symbiotic relationships in these insects. The absence of cellulolytic activity in Panesthia isolates may be explained by several factors. Wood-feeding insects often rely on complex microbial consortia rather than individual bacterial species for lignocellulose degradation (Schapheer et al. 2024). Comparison with other insect gut microbiomes reveals both similarities and unique features. Studies on termite gut bacteria have identified similar genera including *Bacillus* and *Pseudomonas* as major cellulolytic contributors (Bautista-Cruz et al. 2024). However, finding of current study showed strong cellulolytic activity in *Flavobacterium* (cellulolytic index: 2.45), which contrasts with previous reports where this genus typically showed lower cellulolytic potential (Jing et al. 2020). The cellulolytic indices observed was (0.08-3.08), comparable to those reported for termite gut bacteria (0.5-2.8) but higher than those typically found in beetle gut microbiomes (0.2-1.5) (Li et al. 2023). These differences in enzymatic capabilities may be attributed to strain-specific variations and the unique selective pressures within the cockroach gut environment (Schwarz et al. 2023).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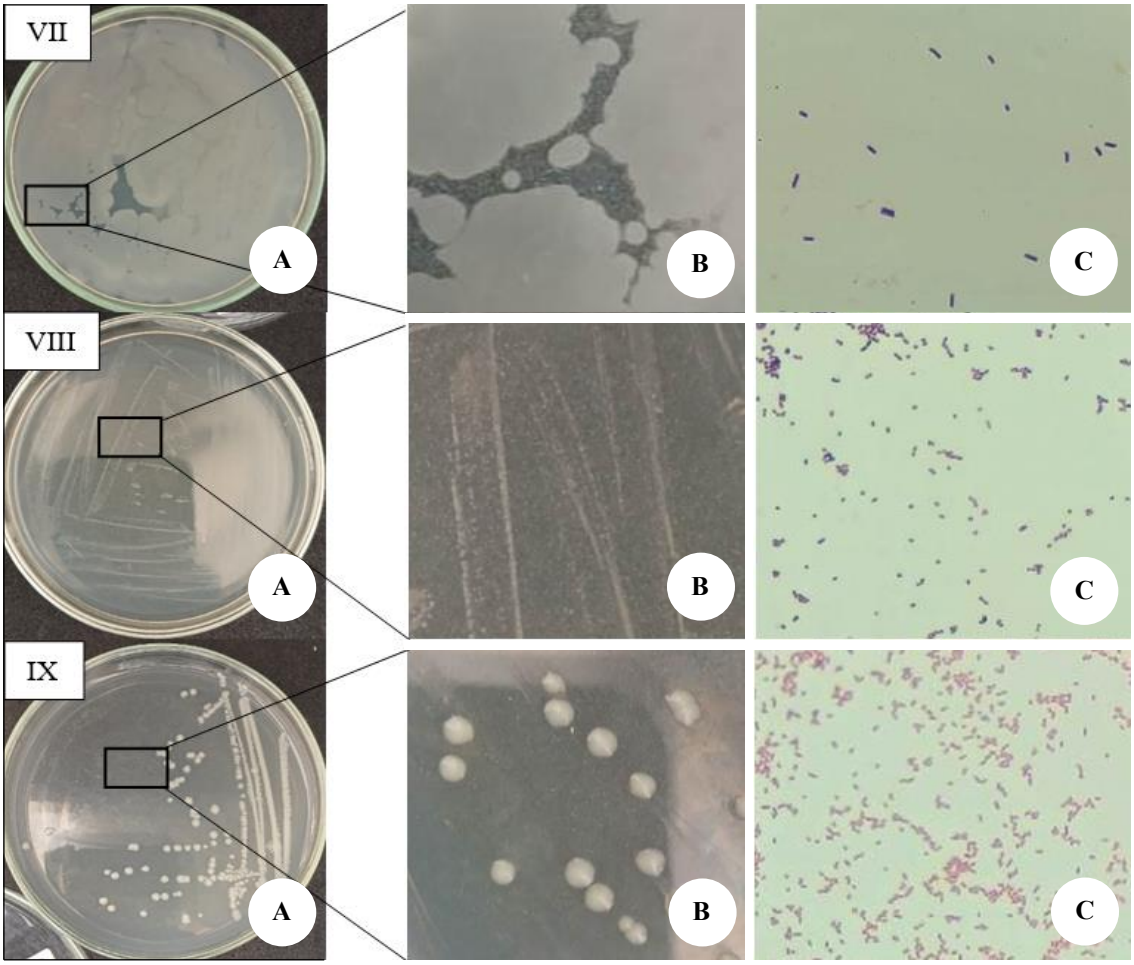

Figure 4. Bacterial morphology of isolates *Panesthia* at the Soraya Research Station, Subulussalam, Aceh, Indonesia. I. BKP-A1, II. BKP-A2, III. BKP-A3, IV. BKP-B1, V. BKP-B2, VI. BKP-B3, VII. BKP-B4, VIII. BKP-C1, IX. BKP-C2. A. Colony morphological observation with their B. Magnification, and C. Gram-staining observed under microscope with 1000× magnification

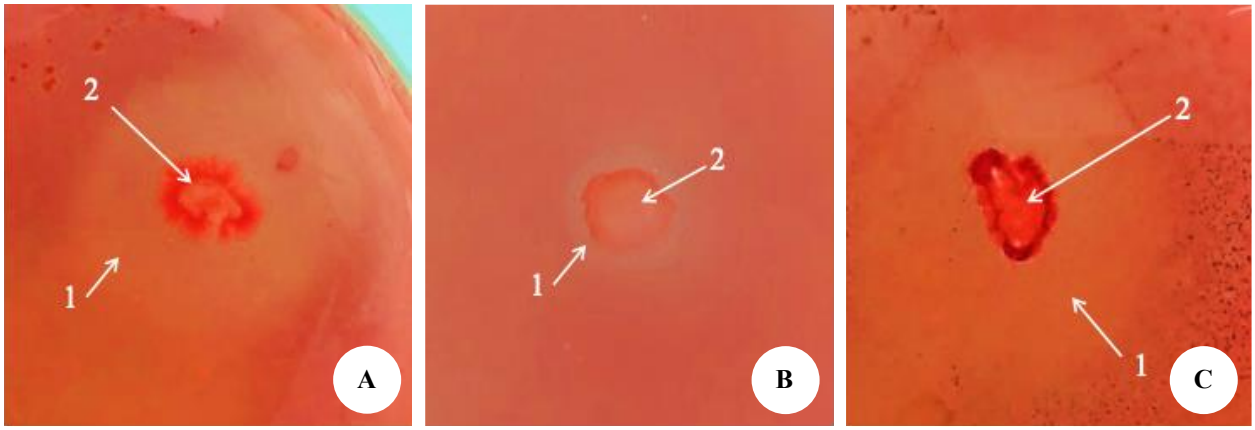

Figure 5. Activity of cellulose degrading isolates with: 1. clear zone, 2. bacterial isolates among the selected isolates of: A. BKB-B1, B. BKB-C1, and C. BKB-C2 from giant cockroaches (Blaberidae) at the Soraya Research Station, Subulussalam, Aceh, Indonesia

The enzymatic mechanisms underlying cellulose degradation likely involve multiple cellulase components. Cellulolytic bacteria typically produce endoglucanases (EC 3.2.1.4) that cleave internal β-1,4-glycosidic bonds, exoglucanases (EC 3.2.1.91) that remove cellobiose units from chain ends, and β-glucosidases (EC 3.2.1.21) that hydrolyze cellobiose to glucose. The varying cellulolytic indices among the present isolates suggest different enzyme

profiles, with *Pseudomonas* likely producing a more complete cellulase system compared to *Bacillus*.

From an ecological perspective, these findings contribute to understanding the role of insect gut bacteria in forest nutrient cycling. Forest cockroaches process approximately 2-5% of leaf litter in tropical forests, and their gut bacteria facilitate the breakdown of recalcitrant cellulose into bioavailable nutrients (Hedjouli et al. 2021). In Aceh's unique forest ecosystem, where rapid decomposition is essential for maintaining soil fertility in nutrient-poor tropical soils, these insect-microbe partnerships may be particularly significant for ecosystem functioning. The presence of efficient cellulolytic bacteria in forest cockroaches suggests their importance in organic matter decomposition, supporting recent research on insect-microbe contributions to ecosystem functioning. These interactions may be particularly significant in tropical forests, where rapid decomposition is crucial for nutrient cycling and maintaining the high productivity of these ecosystems despite poor soil conditions.

The descriptive approach to GC-MS analysis focused on metabolite identification rather than quantitative correlation with cellulolytic activity. The high concentrations of linoleic acid (9,12-octadecadienoic acid) in all three cellulolytic isolates suggest potential biotechnological applications. The high concentration of 9,12-octadecadienoic acid- in the cellulolytic isolates represents a particularly important finding. Recent research has highlighted this compound's importance in various biological processes, such as insecticides (Liu et al. 2023), anticancer agent and antimicrobial agents (Alghonaim et al. 2023), antioxidants (Reza et al. 2021), and potential industrial applications (Mirmohammadsadegh et al. 2021). The differential production levels observed among our isolates, *Bacillus* (67.98%), *Flavobacterium* (56.96%), and *Pseudomonas* (56.05%), suggest distinct metabolic capabilities that could be exploited for biotechnological purposes. Strain-specific metabolic profiles revealed by GC-MS analysis indicate diverse biochemical pathways beyond cellulose degradation. The presence of unique compounds, such as 2,3-butanediol in *Bacillus* and 6-nitro-cylohexadecane-1,3-dione in *Flavobacterium* and *Pseudomonas*, suggests specialized functions within the gut ecosystem. These metabolic signatures align with recent findings on the complex chemical communication networks in insect-microbe symbioses (Seddiqi et al. 2021). The implications of these specialized metabolites extend to broader contexts of gut microbiome interactions, assisting in navigating competitive environments and fostering unique metabolic collaborations among diverse bacterial species (Asnicar et al., 2021; Tu et al., 2020).

The dual functionality of bacterial isolates, combining cellulolytic activity with valuable metabolite production, presents opportunities for integrated bioprocessing approaches. The high cellulolytic activity of bacteria can be used as a source of cellulase enzymes in multiple sectors of industry. This cellulose-degrading enzyme can be used for the extraction of washing powder, fruit and vegetable juices, and starch processing. In addition, it is also used in the textile industry for cotton softening and denim finishing, in detergents for color treatment and cleaning, in the food industry for mashing, and in the pulp and paper industry for drainage improvement and fiber modification (Ejaz et al. 2021). Cellulose enzymes from bacteria are also used in the biomedical field as ingredients in facial masks and pharmaceuticals such as in the manufacture of wound dressings, emulsion stabilizers and tissue engineering (Seddiqi et al. 2021). Moreover, the bioethanol production potential is particularly promising, as these bacteria could facilitate the conversion of agricultural waste and forest residues into biofuels, contributing to sustainable energy solutions (Guddaraddi et al. 2023).

In conclusion, this study successfully isolated and characterized three cellulolytic bacteria from *Blaberus* cockroaches, each exhibiting distinct cellulose-degrading capabilities and significant concentrations of linoleic acid. These results enhance the understanding of insect gut symbionts and their enzymatic potential, while demonstrating promise for industrial applications in enzyme production, agricultural waste management, and bioconversion processes. The dual functionality of these isolates can be used for integrated bioprocessing approaches in industrial and medical applications, while providing insights into tropical forest ecosystem functioning and the complex symbiotic relationships within forest cockroach gut microbiota.

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