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RESEARCH ARTICLE OPEN ACCESS

Grasshopper *(Oxya chinensis)* Gut Bacteria and their Cellulolytic Activity

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Abstract

Lignocellulose is a core component of plant biomass and the most abundant carbohydrate polymer in nature. It is cheap and renewable and has several potential applications; however, it remains underutilized because of its recalcitrance to degradation. Cellulolytic microbes have been found in the gut of herbivorous insects, such as grasshoppers. This study aimed to isolate lignocellulolytic bacteria from the gut of grasshoppers (*Oxya chinensis*) and determine their diversity and potential biomass-degrading activity. A total of 27 culturable isolates were obtained from the grasshopper foregut, midgut, and hindgut. The bacteria hydrolyzed cellulose and lignin, as indicated by a cellulolytic index of 0.12–1.23 and ligninolytic index of 0.1–1.47. Five potential cellulolytic bacterial isolates were selected. Based on 16S rRNA sequencing, the isolates were identified as *Bacillus wiedmannii* (foregut), *Bacillus marcorestinctum*, *Bacillus halotolerans* (midgut), *Paenibacillus zanthoxyli*, and *Bacillus hominis* (hindgut). The highest specific cellulolytic activity (0.0068 U/mg) was detected in *B. wiedmannii* (OCF2), which could be exploited as a potential source of cellulases.

Keywords: *Bacillus*, Cellulolytic Bacteria, Enzymatic Activity, Lignocellulosic Biomass

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INTRODUCTION

Lignocellulosic biomass is the most abundant carbohydrate polymer in nature and one of the cheapest renewable resources. Lignocellulose is a fundamental component of plants and is extensively exploited by industries such as pharmaceutical, biofuels, and food production.[1,2] Lignocellulose is comprised of cellulose (40%–60%), hemicellulose (20%–40%), and lignin (10%–25%).[3] Cellulose constitutes the primary constituent of the cellular wall in plants. It is water-insoluble, with a fibrous, stringent, crystalline form, and a renewable source of energy in the biosphere.[4,5] Hemicellulose consists primarily of xylan, which comprises a main chain of β-1,4-linked xylopyranose residues.[3,6] Lignin, an aromatic biopolymer, is widely prevalent in nature and constitutes approximately 30% of the mass found in the secondary wall of plants.[7]

Lignocellulosic biomass can be hydrolyzed either chemically or enzymatically. The existence of symbiotic bacteria in the digestive tract allows invertebrates and herbivorous animals to digest lignocellulose. Lignocellulolytic enzymes in insects can be derived from gut bacterial symbionts, fungi or the host organism itself.[8] The gut microbes has the ability to break down lignocellulose to release glucose and other fermentable sugars from complex polysaccharides in the plant cell walls.[9] Cellulases are extracellular enzymes capable of converting cellulose to simple sugars[10] by breaking 1,4-β-glycosidic bonds in cellulose, cellodextrin, cellobiose, and other cellulose derivatives.[11,12]

The use of bacteria as a source of cellulose-degrading enzymes has several advantages, namely low production costs, rapidity, simplicity, consistent output, and easy control.[13] Insects possess the ability to produce digestive enzymes through the assistance of symbiotic bacteria residing within their bodies. This mechanism facilitates the efficient breakdown of food and acquisition of energy necessary for their personal growth and developmental processes.[14] Microbes found in the gut of host organisms thrive on lignocellulosic biomass as their major feed.[15] Numerous phytophagous insects, including termites, beetles, and wasps, have highly effective mechanisms for converting lignocellulose.[16] Some groups of arthropoda, such as millipede, have been reported unable to degrade all lignocellulose components. The cellulolytic enzyme was not detected in the insect has amylase activity.[17]

The gut microbiota of grasshoppers is thought to content various type microbes, helping these animals to extract nutrients from plant material.[18] Several bacterial phyla have been documented in the gastrointestinal tract of grasshoppers, including Firmicutes, Proteobacteria, and Actinobacteria.[19] Bacteria are believed to possess lignocellulolytic activities under both aerobic and anaerobic conditions.[20] Some of the anaerobic bacteria include *Acetivibrio*, *Bacteroides*, and *Clostridium*; whereas the aerobic ones include *Bacillus* sp., *Cellulomonas* sp., and *Pseudomonas*.[21] Microbes found in the insect gut, including *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Paenibacillus*, are known to produce lignocellulosic enzymes.[22] Complex interactions exist between gut microbes and their insect hosts to the benefit of both parties.[8] Cellulolytic bacterial isolates were previously reported in the gut of the grasshoppers *Oxya chinensis* and *Oxya velox*.[23,24] The present study aimed to explore biomass degrading activity of bacteria isolated from the gut of *O. chinensis*.

MATERIALS AND METHODS

Sample collection

Four grasshoppers were collected with a net in the agricultural area of Medan Selayang sub-district, Indonesia. The collected grasshoppers were at the 4th instar or higher stage.[17] The grasshoppers were placed in containers, where they had no access to food until dissection and identification in the laboratory.[3]

Isolation of bacteria from the grasshopper gut

Prior to gut dissection, grasshoppers were surface-sterilized with 70% ethanol and kept on ice for 15 min. The entire intestinal tract was removed and divided into three parts: foregut, midgut, and hindgut.[3] The contents of each part were suspended in water and serially diluted to 10^{-6}. At each dilution, 0.1 mL was taken and inoculated in a Petri dish containing nutrient agar medium.[25] The dishes were incubated at 37°C for 24 h, and the number of bacterial colonies was counted. The isolates were characterized both macroscopically and microscopically.[26]

Screening for cellulolytic bacteria

Screening for cellulolytic activity of isolates was done following previously described method.[27] Briefly, they were grown in carboxymethyl cellulose (CMC) agar medium containing 1.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L NaCl, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.01 g/L $MnSO_4 \cdot H_2O$, 0.3 g/L NH_4NO_3, 10.0 g/L CMC, and 12.0 g/L agar. Each bacterial isolate was inoculated at the center of the plate, and the plate was incubated at ± 32°C for 3 days. Subsequently, the dish was flooded with Congo red (0.1% w/v), left covered for 15 min, rinsed with 1 M NaCl, and allowed to stand for 15-20 min.[28] All experiments were performed in triplicate. The clear zone formed around the colony was measured with a caliper and was used to calculate the cellulolytic index.

$$\text{Cellulolytic index} = \frac{\text{(Diameter of clear zone-Diameter of bacterial colony)}}{\text{(Diameter of bacterial colony)}}$$

Screening for ligninolytic bacteria

Ligninolytic activity was screened as described previously.[29] Briefly, the isolates were grown on medium containing 5 g/L glucose, 5.0 g/L yeast extract, 2 g/L KH_2PO_4, 2 g/L Na_2HPO_4, 0.05 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $CaCl_2 \cdot 2H_2O$, 0.01 g/L $CuSO_4 \cdot 5H_2O$, and 15.0 g/L agar. Following sterilization, methylene blue to a final concentration of 1 g/L was added to the medium. Each isolate was inoculated on the agar plate, and the plate was incubated at 32°C for a week. The clear zone formed around the colony was measured with a caliper and was used to calculate the ligninolytic index, based on the same formula used for the cellulolytic index.

Xylanolytic activity was screening as described previously.[6] Each isolate was spot-inoculated on xylan agar medium containing 0.5% (w/v) birch wood xylan, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.02% (w/v) $MgSO_4.7H_2O$, 0.1% (w/v), K_2HPO_4, and 2.0% (w/v) agar. Each bacterial isolate was inoculated in the center of the plate, and the plate was incubated at ± 32°C for 3 days. Subsequently, the dishes were flooded with 0.4% Congo red and, after 10 min, rinsed with 1 M NaCl.[30] The clear zone surrounding the colony was measured with a caliper and was used to calculate the xylanolytic index, based on the same formula used for the cellulolytic index.

Cellulase production

Enzyme production was assessed by inoculating selected bacterial isolates into CMC liquid medium containing basal salt medium without agar (1.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L NaCl, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.01 g/L $MnSO_4 \cdot H_2O$, 0.3 g/L NH_4NO_3), followed by incubation at 37°C with aeration at 120 rpm for 8 days.[31] Subsequently, 5 mL of culture medium suspension was centrifuged for 10 min at 6,000 rpm and 4°C. The supernatant served as a crude extract for quantifying enzyme activity and dissolved protein levels.[28]

Cellulase activity assay

Cellulolytic activity was measured following the modified method of Miller (1959).[32] Briefly, 1 mL crude extract enzyme was pipetted to 1 mL of 1% 3,5-dinitrosalicylic acid (CMC), and incubated for 60 min at 37°C. Following the incubation 1 mL of 20 mM phosphate buffer (pH 7) and 2 mL DNS were added to the mixture. The sample mixture was vortexed and placed in a water bath at 100°C for 10 min. The blank contained 1 mL distilled water instead of crude extract. The absorbance was recorded at 540 nm.[15] One unit of enzymatic activity was defined as the amount of enzyme that produced 1 µmol of glucose per mL per min.[33] A standard curve with glucose was plotted to calculate cellulase activity in the sample. Protein content was measured following Lowry's method at 595 nm and bovine serum albumin was used as a standard.[5] The calculated enzyme level was then used to determine the specific activity of the enzyme based on the following formula:[11]

$$\text{Specific Activity (U/mg)} = \frac{\text{(Enzyme Activity)}}{\text{(Protein Content)}}$$

Molecular identification of potential bacteria

Five cellulolytic isolates with higher cellulolytic indexes were characterized based on 16S rRNA sequences. DNA was isolated by freezing and thawing. One inoculation loop of bacterial suspension was transferred to Eppendorf tube with 100 ml of sterile double distilled water. The tube was chilled (-10°C) for 10 minutes and thawed

Table 1. Morphological characteristics of bacterial isolates from the grasshopper gut

Isolate code	Bacterial colonies				Cell Shape/ Gram stain
	Shape	Color	Margin	Elevation	
			Foregut		
OCF1	Irregular	Pale white	Undulate	Raised	Bacilli / (−)
OCF2	Circular	White	Entire	Flat	Bacilli / (+)
OCF3	Circular	Pale white	Entire	Umbonate	Bacilli / (−)
OCF4	Irregular	Pale white	Undulate	Raised	Bacilli / (+)
OCF5	Irregular	Pale white	Undulate	Raised	Bacilli / (+)
OCF6	Irregular	White transparent	Undulate	Umbonate	Bacilli / (+)
OCF7	Irregular	Red	Entire	Raised	Bacilli / (+)
OCF8	Irregular	Red	Entire	Raised	Cocci / (−)
OCF9	Irregular	Pale white	Entire	Raised	Bacilli / (+)
			Midgut		
OCM1	Irregular	Pale white	Undulate	Raised	Bacilli / (+)
OCM2	Circular	Pale white	Entire	Raised	Cocci / (+)
OCM3	Irregular	White	Undulate	Raised	Cocci / (−)
OCM4	Circular	Yellow white	Entire	Convex	Bacilli / (+)
OCM5	Circular	Red	Entire	Convex	Cocci / (−)
OCM6	Irregular	White transparent	Entire	Umbonate	Bacilli / (+)
OCM7	Irregular	Pale white	Undulate	Umbonate	Bacilli / (+)
OCM8	Circular	Yellow white	Entire	Raised	Bacilli / (+)
OCM9	Irregular	Pale white	Entire	Convex	Bacilli / (+)
OCM10	Circular	Pale white	Entire	Convex	Bacilli / (+)
			Hindgut		
OCH1	Irregular	White	Entire	Raised	Bacilli / (+)
OCH2	Circular	Pale white	Entire	Convex	Bacilli / (+)
OCH3	Irregular	White	Entire	Raised	Bacilli / (+)
OCH4	Irregular	Pale white	Entire	Raised	Bacilli / (+)
OCH5	Circular	White transparent	Entire	Umbonate	Bacilli / (+)
OCH6	Irregular	Pale white	Undulate	Umbonate	Bacilli / (+)
OCH7	Irregular	White	Entire	Umbonate	Bacilli / (+)
OCH8	Circular	Yellow white	Entire	Raised	Bacilli / (+)

Gram stain: +, gram-positive; −, gram-negative

for 10 minutes at 90°C. The step was repeated five times.[34] The suspension was centrifuged at 10,000 x *g* for 10 min. The supernatant was kept at 4°C. The isolated DNA was used as a template for the amplification of the 16S rRNA gene using the bacterial universal primers 27F (5'-AGAGTTTGATCCTGGTCCAG-3') and 1492R (5'-CTACGGGCTACCTTGTTACGA-3').[35] The reaction was prepared in a total volume of 25 µL with the following program: pre-denaturation at 94°C for 2 min; 40 cycles of denaturation at 92°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min; and post-elongation at 72°C for 5 min.[34] The PCR results were run along with a 1-kb DNA marker on a 1% agarose gel at 80 V and 400 mA for 60 min for monitoring the results. The PCR product was sent to Macrogen Singapore for sequencing.

Bioinformatics analysis

The nucleotide sequence of the 16S ribosomal DNA (rDNA) gene from the five selected isolates was aligned and compared with 16S ribosomal RNA (rRNA) data available in GenBank using the BLASTn tool.[36] Analysis of

bacterial kinship based on phylogenetic trees was performed in MEGA XI software with 1,000 bootstrap repetitions.[37,38]

RESULTS AND DISCUSSION

Bacterial isolates from the grasshopper gut

The diversity and role of bacteria resides on the digestive system of animals has been studied from different viewpoints, and different approaches have been done to isolate types of microorganism. Koubova *et al.*[39] isolated actinobacteria, bacteria and fungi from millipede *Telodeinopus aoutii* gut. Willis *et al*. [40] isolated bacteria from the foregut and hindgut of the Carolina wasp *Dissosteira carolina*. Here, bacteria were successfully isolated from all three parts of the grasshopper gut, yielding a total of 27 culturable isolates: 9 from the foregut, 10 from the midgut, and 8 from the hindgut. Previously, Shil *et al*.[23] recovered and identified 15 bacterial isolates from the gut of *O. velox*. Abdullah *et al*. [41] successfully isolated 80 bacterial types from *Valanga nigricornis*. Wang *et al*.[42] isolated bacteria from three different grasshoppers: *Aiolopus tamulus* (31 isolates), *Oedaleus decorus asiaticus* (32 isolates), and *Shirakiacris shirakii* (30 isolates).

Based on the macroscopic characterization reported in Table 1, the bacterial colonies presented mostly an irregular shape, milky white color, complete margin, and raised elevation. Gram staining revealed 22 gram-positive bacteria and 5 gram-negative bacteria. The different shape and growth of each isolate indicated that they originated from different types of bacteria.[43] The highest number of isolates was obtained from the midgut and the smallest from the hindgut. According to Arfah *et al*.,[11] many digestive processes, such as the absorption of carbohydrates, proteins, and lipids, occur in the midgut of grasshoppers; however, the distribution of bacteria in the foregut and midgut differs between that of *O. chinensis* and *Oedaleus infernalis*.[44]

Lignocellulolytic activity of bacterial isolates

Clear zones were visible around inoculation sites on CMC agar plates flooded with 1% Congo red (Figure 1A) and methylene blue-containing lignin agar plates (Figure 1B). Saini *et al*.[45] stated that the clear zone around a colony indicated extracellular cellulase production by bacteria. Congo red at 1% enables the detection of clear zones hydrolyzed by cellulase.[46] Xylanolytic activity was not visible in xylan agar, as no clear zone appeared around the bacterial inocula after flooding the plate with 0.4% Congo red. This finding suggests that the *O. chinensis* gut bacteria probably cannot utilize the carbon in xylan agar. Alternatively, the screening method employed in this study[6,30] may not be suitable for detecting xylanase activity in bacterial samples.

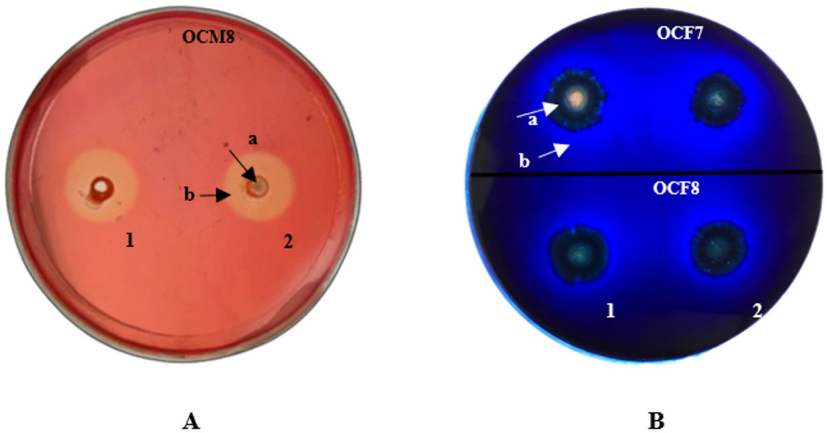

Figure 1. Presence of (A) cellulolytic activity in carboxymethyl cellulose agar and (B) lignocellulolytic activity in lignin agar. 1: first repetition; 2: second repetition; a: bacterial colonies; b: clear zone.

Indeed, while xylanase production in fungi is well documented,[3] little is known about such enzymes in the insect gut.

The formation of clear zones on methylene blue agar plates indicated that the bacterial colony produced enzymes capable of degrading lignin.[43] Dineshkumar *et al*.[46] suggested the use of methylene blue to quantify ligninolytic activity. The decolorization of methylene blue is due to the secretion of extracellular ligninolytic enzymes.[47] Isolates that can form a clear zone with a diameter twice that of the colony are considered potential producers of ligninolytic enzymes.[48]

The enzymatic activity of the different bacterial isolates was calculated based on measuring the diameter of the clear zones around the colonies (Table 2) and the values were the average of three measurements. Bacterial isolates possessed cellulolytic (100%) and ligninolytic (55.5%) activity, but no xylanolytic (0%) activity, suggesting a varying ability to hydrolyze the carbon source. The lignocellulolytic index is strongly influenced by the ability to produce the corresponding enzymes.

The highest cellulolytic index was found in isolates OCH7 (1.23), OCM8 (1.20), and OCM4 (1.07); the highest ligninolytic index was detected in isolates OCF7 (1.47), OCF3 (1.31), and OCM8 (1.22). Notably, the xylanolytic index was zero in all isolates (Table 2). Hence, the bacteria found in

Table 2. Lignocellulolytic index of bacterial isolates from the grasshopper gut

Isolate code	Cellulolytic index	Ligninolytic index	Xylanolytic index
OCF1	0.45	0	0
OCF2	0.86	1.04	0
OCF3	0.30	1.31	0
OCF4	0.71	0	0
OCF5	0.13	0.99	0
OCF6	0.29	0.71	0
OCF7	0.61	1.47	0
OCF8	0.32	0.32	0
OCF9	0.37	0	0
OCM1	0.41	0.64	0
OCM2	0.41	0	0
OCM3	0.12	1.02	0
OCM4	1.07	0	0
OCM5	0.12	0.65	0
OCM6	0.50	0	0
OCM7	0.20	0.4	0
OCM8	1.20	1.22	0
OCM9	0.60	0	0
OCM10	0.50	0.54	0
OCH1	0.37	0	0
OCH2	0.42	0	0
OCH3	0.53	0	0
OCH4	1.06	0.1	0
OCH5	0.99	0.16	0
OCH6	0.34	0.17	0
OCH7	1.23	0	0
OCH8	0.91	0	0

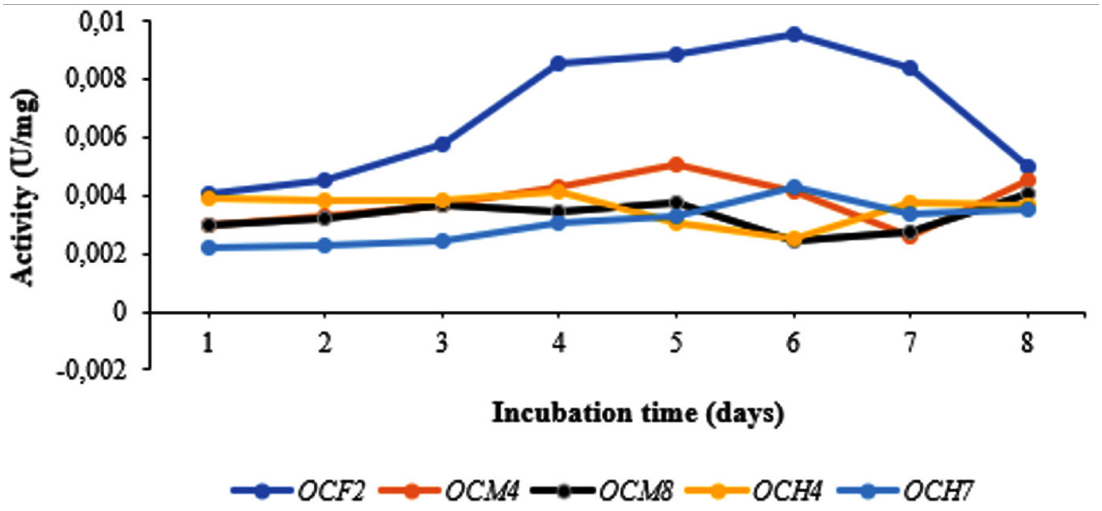

Figure 2. Specific cellulase activity for selected bacterial isolates

the gut of *O. chinensis* might be unable to use xylan as a carbon source but can readily utilize cellulose and lignin.

Jimenez and Hernandez[49] reported that fungi showed greater capability for degrading lignocellulosic materials than bacteria in the gut of wood-feeding Coleoptera, with the genera *Trichoderma*, *Bionectria*, and *Trametes* showing positive results in all the assays performed. Abdullah *et al*.[41] reported a cellulolytic index of 0.9-1.7 for bacterial isolates from the grasshopper *V. nigricornis*. Ferbiyanto *et al*.[19] isolated bacteria from the gut of the termite *Macrotermes gilvus* and reported a cellulolytic index of 0.75–2.5. Simol *et al*.[21] reported 10 potential ligninolytic microbes from the gut of the termite *Coptotermes curvignathus*. Recent work by Koubova *et al*.[39] found that 30% of isolated bacteria and fungi from the gut of millipede *T. aoutii* exhibited cellulase activity in vitro. They also identified high activity of cellulase from Actinobacteria *Streptomyces* and *Kitasatospora*.

Cellulase activity of bacterial isolates

Bacterial isolates with the highest cellulolytic index on plates were incubated in liquid CMC medium for 8 days. Subsequently, cellulase activity, protein level, and specific cellulase activity were measured. As shown in Figure 2, in general, the highest specific cellulase activity was detected on day 5, particularly for OCF2 (0.0088 U/mg) and OCM4 (0.0050 U/mg); the values for OCM8, OCH4, and OCH7 clustered around 0.0037 U/mg, 0.0030 U/mg, and 0.0032 U/mg, respectively. The specific cellulase activity increased in some cases until day 6 and decreased significantly thereafter. The protein level ranged from 88.829 mg/mL to 92.4 mg/mL. Grass-consuming grasshoppers and the wood-consuming wood borer possess higher gut cellulase activities than leaf-consuming silkworms.[50] Specifically, cellulase activity in the gut fluid of *O. velox* was reported as 0.759 ± 0.005 U/mg,[3] whereas that of *Tribolium castaneum* larvae was 0.016 U/mg.[51] Sreena *et al*.[52] isolated

Table 3. BLASTn of the 16S rRNA gene from five bacterial isolates

Isolate code	Closely related bacteria	GenBank accession no.	Identity (%)
OCF2	*Bacillus wiedmannii* strain FSL W8-0169	NR_152692.1	93.46
OCM4	*Bacillus marcorestinctum* strain LQQ	NR_117414.1	94.03
OCM8	*Bacillus halotolerans* strain CR-119	NR_115283.1	86.15
OCH4	*Paenibacillus zanthoxyli* strain JH29	NR_043876.1	97.37
OCH7	*Bacillus hominis* strain BML-BC059	NR_175557.1	95.79

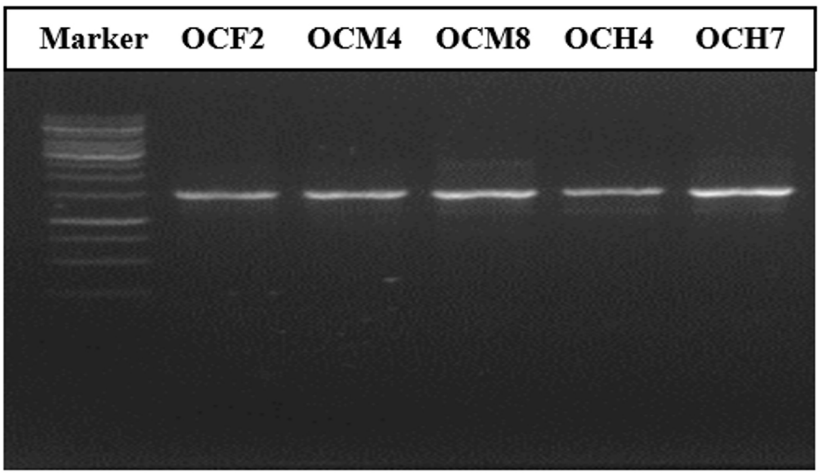

Figure 3. PCR results showing amplification of the 16S rRNA gene. Row 1 corresponds to the 1-kb DNA marker. Rows 2–5 correspond to bacterial isolates from the gut of *O. chinensis*

five strains of cellulolytic bacteria from two termite species and found that the highest endoglucanase activity was that of *Bacillus cereus* (5.06 U/mg) while the lowest was 2.98 U/mg.

Molecular identification of bacterial isolates

Molecular identification of the bacterial isolates was performed by sequencing their 16S rRNA gene using universal primers. Based on the results in Figure 3, the amplified DNA was 1,500 bp in size. The 16S rRNA PCR products of five potential cellulolytic microbes were purified, sequenced, and aligned for comparison by BLASTn against the NCBI database (Table 3).

Alignment revealed that the cellulolytic bacteria belonged to two genera: *Bacillus* and *Paenibacillus*. Each strain is considered to fit one genus, species, or strain if the similarity index value obtained meets the criteria. Based on Table 3, the five species of cellulolytic bacteria from the isolates belonged to the phylum Firmicutes: *Bacillus wiedmannii*, *Bacillus marcorestinctum*, *Bacillus halotolerans*, *Paenibacillus zanthoxyli*, and *Bacillus hominis*. The results of this study are in accordance with the findings of Wang *et al*.[42] who isolated and screened five cellulose-degrading isolates from the gut of the grasshopper *Yunnanacris yunnaneus.*

The phylogenetic tree of the cellulolytic bacterial isolates with 1,000 bootstrap repetitions is shown in Figure 4. The cellulolytic bacteria isolated from the gut of the grasshopper *O. chinensis* were closely related to *B. wiedmannii* FSL W8-0169, as indicated by an identity of 93.46%. Chantarasiri[53] identified cellulolytic bacteria from freshwater wetlands and found *B. wiedmannii* strain W1401 in one of the isolates. Danial *et al*.[54] reported that *B. wiedmannii* isolated from a cattle manure sample utilized sugar fruit peel waste efficiently to produce polyhydroxybutyrate.

Another isolate exhibited 94.03% identity to *B. marcorestinctum* strain LQQ. The latter was isolated from soil samples by Han *et al*.,[55] who identified it as gram-positive, facultative anaerobic, rod-shaped bacterium. Effendi *et al*. [56] reported that *B. marcorestinctum* strain LQQ functioned as a biocontrol agent against spoilage in plants. Zhong *et al*.[57] described the effect of *B. marcorestinctum* as a starter, which could improve the quality and safety of Yibin Yacai by changing the microbial community during fermentation.

A third isolate displayed 86.15% identity with *B. halotolerans* strain CR-119. Ouertani *et al*. [58] isolated *B. halotolerans* from a tannery wastewater and identified it as a keratinolytic bacterium. Yousef *et al*.[59] recovered *B. halotolerans* from saline mud samples and demonstrated potential cellulase production. Wen *et al*.[60] reported that *B. halotolerans* produced extracellular alkaline proteases, the activity of which was the highest after 12 h of incubation.

A fourth isolate exhibited 86.15% identity with *P. zanthoxyli* strain JH29. Ma *et al*.[61] identified *P. zanthoxyli* as a novel nitrogen-fixing species

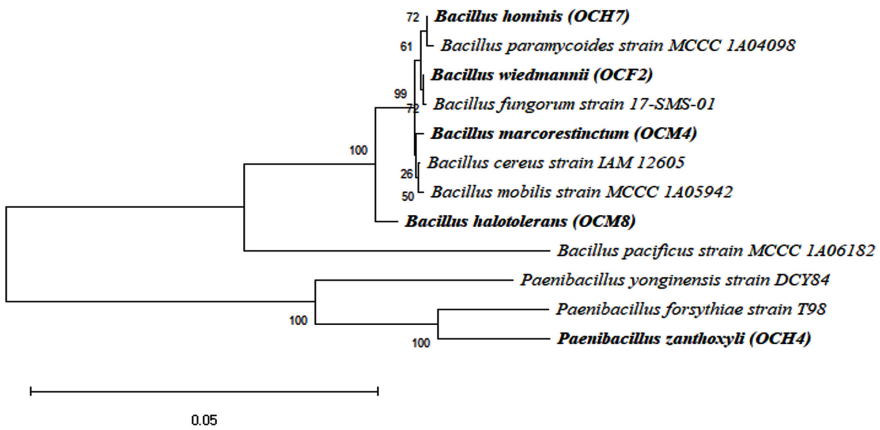

Figure 4. Phylogenetic tree of potential bacteria from the grasshopper gut

isolated from the rhizosphere of *Zanthoxylum simulans*. The fifth isolate showed 95.79% identity with *B. hominis* strain BML-BC059. Several *Bacillus* species, such as *B. cereus*, *B. licheniformis*, *B. megaterium*, and *B. subtilis*, are known to be effective cellulolytic bacteria.[33] The present study reports, for the first time, that *B. marcorestinctum*, *P. zanthoxyli*, and *B. hominis* possess cellulolytic activity in the grasshopper gut.

CONCLUSION

This study demonstrated the presence of lignocellulolytic activity of bacteria in the gut of *O. chinensis*. The 27 culturable bacterial isolates obtained exhibited cellulolytic and ligninolytic activity. This indicates that bacterial community in the gut of grasshopper plays in digesting food materials. Molecular identification based on the 16S rRNA gene sequence identified the five potential bacterial isolates as belonging to the genus *Bacillus*. The isolate with the highest cellulolytic ability was *Bacillus wiedmannii* (OCF2).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All data generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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