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Diversity of cellulolytic bacteria from *Macrotermes gilvus* gut isolated from Indralaya peatland region, Indonesia

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Abstract. *Oktiarni D, Kasmiarti G, Nofyan E, Miksusanti, Hasanudin, Hermansyah. 2021. Diversity of cellulolytic bacteria from* Macrotermes gilvus *gut isolated from Indralaya peatland region, Indonesia. Biodiversitas 23: 486-495.* The termite *Macrotermes gilvus* Hagen from Tanjung Senai, Inderalaya peatland region, is classified as a high-level termite. Termites like eusocial insects break down cellulosic biomass into glucose by bacteria that produce cellulolytic enzymes in their gut. In the present study, new species of cellulolytic bacteria from *Macrotermes gilvus* Hagen gut were determined by biomolecular assay. Bacterial isolates were isolated and purified by using DNA kit and the optical density of DNA bacterial isolates was obtained by using spectrophotometer nanodrop. A total of 24 bacterial isolates were amplified using PCR to determine the fragment of 16S rRNA gene, performed using BLAST-N program and compared with NCBI database. Result from the sequences of 16S rRNA gene showed that the new species were identify as *Enterobacter cloacae* (isolates 1, 3, 6, 7, 10, 14, 19, 20, 25, 28, 39, 40, 44, 60, and 64), *Klebsiella pneumoniae* (isolates 24, 43, and 62), *Klebsiella quasipneumoniae* (isolates 29, 36, and 37), *Klebsiella varicolla* (isolate 52), *Enterobacter roggenkampii* (isolate 56) and *Enterobacter asburiae* (isolate 59).

Keywords: Biomass, cellulolytic bacteria, cellulolytic enzyme, *Macrotermes gilvus* Hagen, termite

INTRODUCTION

Termites are insects that can degrade cellulose into monosaccharides by cellulolytic enzymes in their guts. Termites are divided into two types, as low-level termites and high-level termites, based on the eukaryotic symbionts in their guts. Low-level termites consist of fungi and bacteria, but high-level termites contain less fungi and only bacteria (Ni and Tokuda 2013). The digestive tunnel of termites contains various bacteria and fungi with cellulolytic enzyme activity, which converts cellulose into simple sugars. Three groups of cellulase enzymes can hydrolyze cellulose, namely endoglucanase, exoglucanase, and β-glucosidase enzymes (Ferbiyanto et al. 2015; Sharma et al. 2015; Yadav et al. 2019; Pabbathi et al. 2021).

There are two termites: reproductive termites (queen/alates: queen) and non-reproductive termites (soldier and worker). Termites usually have one colony, queen termites are responsible for reproducing and guarding eggs, soldier termites protect the colony, while worker termites maintain, repair the colony and deliver food for the queen termites. The digestive tract of termite contains several types of bacteria and fungi (low-level termite: *Coptotermes curvignathus* Holmgren), and some of the digestive tracts contains only bacteria and less of fungi (high-level termite: *Macrotermes gilvus* Hagen) (Ferbiyanto et al. 2015).

Bacteria and fungi isolated from termites are of major interest due to their high-level enzymatic activities, especially cellulolytic activity (Tsegaye et al. 2019). Cellulose as potential biomass can be naturally degraded by the bacteria in termite gut. Almost low-level termites are diet of wood (Hongoh 2011), while high-level termites are diet of lignocellulosic biomass, like wood, plant litter, grass litter, and dirt (Hongoh 2011; Brune 2014; Ferbiyanto et al. 2015; Mikaelyan et al. 2015). Recently, research regarding termite has significantly increased, because of their ability to degrade lignocellulose by bacteria symbionts in their gut, becoming specialist agents to hydrolyze cellulose biomass. Termites are seen as a potential major player in various ecosystem functions, as termites are sensitive to habitat disturbance, resulting in a decrease in diversity. The diversity of termites certainly decreases with increasing land disruption (Neoh et al. 2017; Jalaludin et al. 2018; Handayani and Aji 2020) and peatland areas.

Peatland is a very unique land, during the dry season it dries up and even burn, while during the rainy season it becomes moist and waterlogged. This greatly affects the peatland ecosystem, including fauna that have always adapted to environmental changes that occur every season

(Neoh et al. 2017; Handayani and Aji 2020). The continuously high temperatures of tropical swamp fires and their constantly burning effect have a greater detrimental impact on soil-dwelling, include termite. The diversity of organisms above and below the soil on peatlands is lower than on acid sulphate. Termites are thought to be the primary factor of the circulations of food network in certain tropical peat swamp forests and act as maintain ecological stability system. Very interestingly, termites in peatland have the ability to adapt quickly to the changing climate which also affects their lifestyle and food sources (Neoh et al. 2017; Jalaludin et al. 2018; Handayani and Aji 2020). Indeed, their habitat and diet also affect the various type of bacteria in their digestive tract.

This study aimed to isolate and identify the novel species of cellulolytic bacteria in *Macrotermes gilvus* Hagen gut obtained from Indralaya peatland region, Indonesia. The diversity of cellulolytic bacteria was performed by polymerase chain reaction (PCR) and analysis of 16S rRNA (ribonucleic acid) genes of nucleotide fragments executed by using the Bi-directional DNA (deoxyribose nucleic acid) sequencing method.

MATERIALS AND METHODS

Study area

Termite *Macrotermes gilvus* Hagen was obtained from Tanjung Senai peatland region in Indralaya, Ogan Ilir, South Sumatera province. Termites were prepared and isolated as described by (Oktiarni et al. 2021). Ten of workers termite were sterilized with 70% alcohol for 30 second. Termite digestive tract was aseptically dissected by using micro-tweezers and suspended in 0.85% NaCl. 0.1 mL of the suspension was spread on carboxymethyl cellulose (CMC) agar media at 37°C for 2 days. Bacterial isolates on CMC agar media were taken as much as 10, and scratched on CMC agar media aseptically and incubated at 37°C for 2 days. After incubation, CMC media was stained with 1% (w/v) *Congo red* for 30 minutes or with 1% iodine for 30 minutes at room temperature and decolorized with 1 M NaCl solution for 15 minutes. The screening of bacteria was conducted on CMC agar media. From sixty-four isolates, twenty-four isolates were identified as cellulolytic bacteria. The bacterial isolates from the *M. gilvus* Hagen gut with high cellulolytic index were further determined by biomolecular assay.

Isolation and purification DNA of bacterial isolates

The 16S rRNA genes of 24 bacterial isolates were isolated and purified by using DNA kit Quick-DNA™ Fungal/Bacterial Miniprep (Zymo Research) based on the manufacturer's procedures.

DNA quantification using spectrophotometer nanodrop

1 μL sample of DNA bacterial isolates was transferred to drop plate and measured for optical density ratio of 260/280 (for DNA) and 260/230 (for RNA). The optical density was carried out by using a spectrophotometer nanodrop (Thermo Scientific™ NanoDrop™). 1 µL PCR products were assayed by electrophoresis with 0.8% TBE agarose. The DNA mark was checked by running the DNA into gel agarose and visualized using Cyber Green staining on an ultraviolet transilluminator.

PCR analysis of 16S RNA genes

PCR amplification of 16S RNA genes of 24 purified isolates was conducted with (2x) My Taq HS Red Mix (Bioline, BIO-25048) and using primer universal of 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTA CCTTGTTACGACTT-3). The 16S RNA genes 1024 purified isolates was amplified and measured using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems (USA). Thermocycling was regulated by initial denaturation stage at 94°C for 4 minutes, followed by discontinuation stage 35 cycles at 94°C for 40 seconds, annealing stage at 55°C for 1 minute, multiplication stage at 72°C for 1 minute 10 seconds, and final multiplication at 72°C for 10 minutes.

PCR sequencing product

The DNA extract was sequenced by using BigDye® Terminator v3.1 Cycle Sequencing Kit. The PCR products were sequenced by using the Bi-directional DNA sequencing method. Data from the 16S rRNA gene sequences were analyzed using BLAST-N (basic local alignment search tool) program in the NCBI gene bank (national center for biotechnology information) (http://www.ncbi.nlm.nih.gov).

Phylogenetic tree analysis of amplified 16S RNA genes

The 16S rRNA gene further constructed a phylogenetic tree using software (MEGA 5.05) with neighbor-joining at 1000X bootstrap.

RESULTS AND DISCUSSION

The DNA concentration of bacterial isolates ranged from 4.8-1.4 ng/μL (Table 1), whereas 260/280 nm ratio of bacterial isolates showed around 3.69-1.46, which indicated that all bacterial isolates had high purities. However, data of 260/230 ratio showed about 0.18-0.01, it was the bacterial isolates had significant amount of impurities that will bothering downstream applications, especially for reverse transcription.

The 16S rRNA gene was determined by using PCR method. The fragment of 16S rRNA genes were amplified and conducted by using gel electrophoresis. The purity of the amplified 16S rRNA gene fragment was determined by the only one thick and single band on the electrophoresis gel. The 16S rRNA gene fragments of each bacterial isolates showed only one band around 1400 base pairs (Figure 1). The results showed that only one type of molecule is absent from impurities.

Table 1. DNA concentration of bacterial isolates

Isolates	Concentration (ng/μL)	$A_{260/280}$	$A_{260/230}$
1	3.1	1.76	0.13
3	2.8	1.72	0.18
6	4.2	3.75	0.13
7	2.1	3.45	0.03
10	4.3	1.64	0.08
14	2.8	1.99	0.04
19	1.4	2.69	0.04
20	4.0	2.22	0.09
24	1.6	3.42	0.12
25	2.8	1.92	0.11
28	1.8	2.50	0.05
29	2.1	3.04	0.11
36	1.8	2.24	0.08
37	2.4	2.04	0.02
39	2.9	2.01	0.08
40	2.5	2.05	0.14
43	3.4	2.26	0.04
44	3.7	1.70	0.05
52	2.4	3.21	0.01
56	3.0	2.32	0.12
59	1.4	3.69	0.01
60	2.3	2.62	0.02
62	4.5	1.46	0.07
64	2.2	2.43	0.13

Furthermore, the 16S rRNA gene fragment analyzed for its nucleotide base sequence to determine the species of each bacterial isolate. BLAST-N of 16S rRNA genes of isolates 1, 3, 6, 7, 10, and 14 (Table 2) and the phylogenetic tree of isolates (Figure 2), showed that isolates 1 and 3 had similarity with *Enterobacter cloacae* strain CBG15936 (CP046116.1) with 100% percentage identity. Isolates 6 and 7 had similarity with *Enterobacter cloacae* strain MBB8 (MT138539.1) with 99.88% and 100% percentage identity, respectively. Isolate 10 had also similarity with strain VITGTJ1 (MN853688.1) *Enterobacter cloacae*, with a percentage identity of 99.88%, while isolate 14 had similarity with *Enterobacter cloacae* strain NH77 (CP040827.1) with a percentage identity of 99.86%.

Data from 16S rRNA genes of isolates 19, 20, 24, 25, 28 and 29 (Table 3) and the phylogenetic tree of isolates (Figure 3), showed that isolate 19 had similarity with *Enterobacter cloacae* strain CBG15936 (CP046116.1) with 100% percentage identity. Isolates 20 and 25 had similarity with *Enterobacter cloacae* strain FDAARGOS 1431 (CP077211.1) and *Enterobacter cloacae* strain NH77 (CP040827.1) with 100% percentage identity. Moreover, isolates 24 and 28 had similarity with *K. pneumoniae* strain B16KP0141 (CP052537.1) with percentage identity of 99.86% and 99.93%, respectively, while isolate 29 had similarity with *K. quasipneumoniae* strain HKUOPL4 (CP014156.1) with 100% percentage identity.

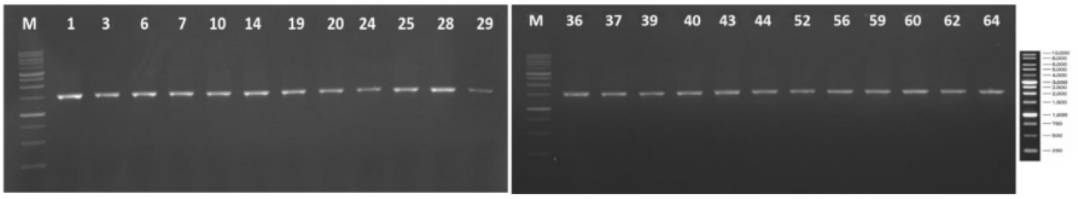

Figure 1. Profile of PCR product from bacterial isolates fragment of 16S rRNA gene on electrophoresis gel.

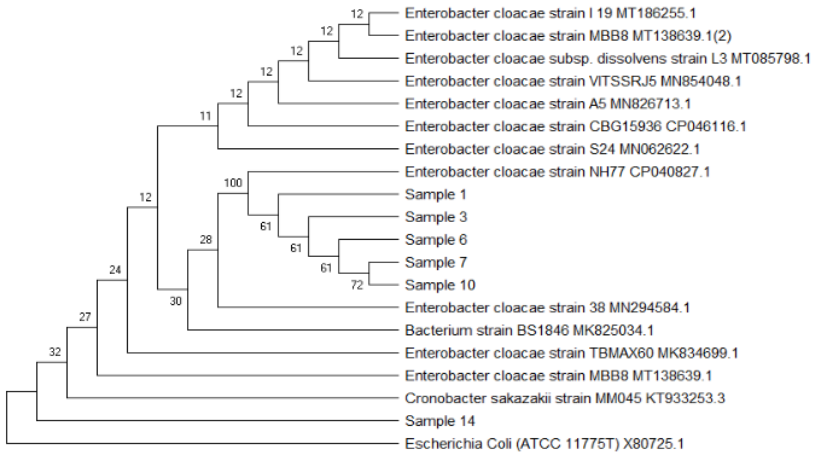

Figure 2. Dendrogram of bacterial isolates 1, 3, 6, 7, 10, and 14

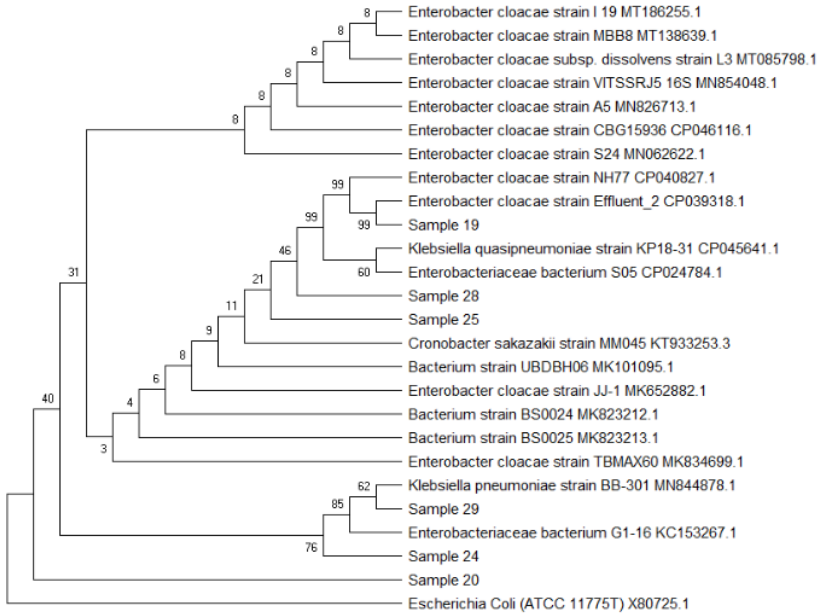

Figure 3. Dendrogram of bacterial isolates 19, 20, 24, 25, 28, and 29

Table 2. BLAST-N of 16S rRNA genes of isolates 1, 3, 6, 7, 10 and 14

Isolates	Accession number	Species	Max score	Total score	Query coverage	E value	Percent. identity	Accession length
1	MT138639.1	*Enterobacter cloacae* strain MBB8	1661	1661	100%	0	100%	1550
	MN854048.1	*Enterobacter cloacae* strain VITSSRJ5	1661	1661	100%	0	100%	1556
	CP046116.1	*Enterobacter cloacae* strain CBG15936	1661	13278	100%	0	100%	5033927
	MN062622.1	*Enterobacter cloacae* strain S24	1661	1661	100%	0	100%	1447
	MK834699.1	*Enterobacter cloacae* strain NH77	1661	1661	100%	0	100%	1422
3	MT138639.1	*Enterobacter cloacae* strain MBB8	1530	1661	100%	0	100%	1550
	MN854048.1	*Enterobacter cloacae* strain VITSSRJ5	1530	1661	100%	0	100%	1556
	CP046116.1	*Enterobacter cloacae* strain CBG15936	1530	12230	100%	0	100%	5033927
	MN062622.1	*Enterobacter cloacae* strain S24	1530	1530	100%	0	100%	1447
	CP040827.1	*Enterobacter cloacae* strain NH77	1530	12219	100%	0	100%	5040532
6	MT507091.1	*Enterobacter cloacae* strain Remi 11	1507	1507	100%	0	99.88%	1412
	MT415400.1	*Enterobacter cloacae* strain S26	1507	1507	100%	0	99.88%	1415
	MT265053.1	*Enterobacter cloacae* strain CMFRI/ECI-02	1507	1507	100%	0	99.88%	1427
	MT186255.1	*Enterobacter cloacae* strain I19	1507	1507	100%	0	99.88%	1481
	MT138539.1	*Enterobacter cloacae* strain MBB8	1507	1507	100%	0	99.88%	1550
7	MT415400.1	*Enterobacter cloacae* strain S26	1507	1507	100%	0	100%	1415
	MT265053.1	*Enterobacter cloacae* strain CMFRI/ECI-02	1570	1570	100%	0	100%	1427
	MT085798.1	*Enterobacter cloacae* strain I19	1570	1570	100%	0	100%	1471
	MT138539.1	*Enterobacter cloacae* strain MBB8	1570	1570	100%	0	100%	1550
	MT186255.1	*Enterobacter cloacae* subso dissolvens str L3	1570	1570	100%	0	100%	1481
10	MN853688.1	*Enterobacter cloacae* subso dissolvens str VITGTJ1	1526	1526	100%	0	99.88%	1599
	KY930709.1	*Enterobacter cloacae* strain NIBSM OsR09	1526	1526	100%	0	99.88%	1500
	KY930712.1	*Enterobacter cloacae* strain NIBSM OsR12	1526	1526	100%	0	99.88%	1491
	MN294584.1	*Enterobacter cloacae* strain 38	1526	1526	100%	0	99.88%	1479
	MK825034.1	*Bacterium* strain BS1846	1526	1526	100%	0	99.88%	1446
14	MT138639.1	*Enterobacter cloacae* strain MBB8	2597	2597	100%	0	99.86%	1550
	CP040827.1	*Enterobacter cloacae* strain NH77	2597	20713	100%	0	99.86%	5040532
	CP039303.1	*Enterobacter cloacae* strain Effluent 4	2597	20569	100%	0	99.86%	5154368
	CP017475.1	*Enterobacter cloacae* strain M12X01451	2597	20680	100%	0	99.86%	4918273
	KT933253.3	*Cronobacter sakazakii* strain MM045	2597	2597	100%	0	99.86%	1507

Table 3. BLAST-N of 16S rRNA genes of isolates 19, 20, 24, 25, 28, and 29

Isolates	Accession number	Species	Max score	Total score	Query coverage	E value	Percent. identity	Accession length
19	MT186255.1	*Enterobacter cloacae* strain I19	1807	1807	100%	0	100%	1481
	MN854048.1	*Enterobacter cloacae* strain VITSSRJ5	1807	1807	100%	0	100%	1556
	CP040827.1	*Enterobacter cloacae* strain CBG15936	1807	14446	100%	0	100%	5033927
	CP046116.2	*Enterobacter cloacae* strain NH77	1807	14434	100%	0	100%	5040532
	MK834699.1	*Enterobacter cloacae* strain TBMAX60	1807	1807	100%	0	100%	1422
20	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2621	20927	99%	0	100%	5316745
	CP056776.1	*Enterobacter cloacae* DSM 30054	2621	20927	99%	0	100%	5316635
	CP001918.1	*Enterobacter cloacae* subso cloacae ATCC 13047	2621	20876	99%	0	100%	5314581
	KX.242266.1	*Enterobacter cloacae* strain OS5.6	2617	2617	99%	0	100%	1511
	KT.933255.3	*Enterobacter sp* MM034	2617	2617	99%	0	100%	1529
24	CP052262.1	*Klebsiella pneumoniae* strain E16KP0288	2601	20682	100%	0	99.86%	
	CP052537.1	*Klebsiella pneumoniae* strain B16KP0141	2601	20770	100%	0	99.86%	
	CP042858.1	*Klebsiella pneumoniae* strain NMI4662	2601	20654	100%	0	99.86%	
	CP045661.1	*Klebsiella pneumoniae* strain SMU18037509	2601	20715	100%	0	99.86%	
	CP030072.1	*Klebsiella pneumoniae* strain DA12090	2601	20687	100%	0	99.86%	
25	CP040827.1	*Enterobacter cloacae* strain NH77	2612	20831	100%	0	100%	
	MK823213.1	*Bacterium* strain BS0025	2612	2612	100%	0	100%	
	CP039303.1	*Enterobacter cloacae* strain Effluent	2612	20787	100%	0	100%	
	KT933253.3	*Cronobacter sakazakii* strain MM045	2612	2612	100%	0	100%	
	CP016906.1	*Enterobacter cloacae* strain isolate SBP-8	2612	1661	100%	0	100%	
28	CP040827.1	*Enterobacter cloacae* strain NH77	2606	20787	100%	0	99.93%	
	MK823213.1	*Bacterium* strain BS0025	2606	2606	100%	0	99.93%	
	CP039303.1	*Enterobacter cloacae* strain Effluent	2606	20743	100%	0	99.93%	
	CP017475.1	*Enterobacter cloacae* strain M12X014451	2606	20754	100%	0	99.93%	
	KT933253.3	*Cronobacter sakazakii* strain MM045	2606	2606	100%	0	99.93%	
29	MN844878.1	*Klebsiella pneumoniae* strain BB-301	2612	2612	100%	0	100%	
	CP035207.1	*Klebsiella quasipneumoniae* strain TH114	2612	20776	100%	0	100%	
	CP014156.1	*Klebsiella quasipneumoniae* strain HKUOPL4	2612	20698	100%	0	100%	
	CP014154.1	*Klebsiella quasipneumoniae* strain HKUOPJ5	2612	20892	100%	0	100%	
	CP014155.1	*Klebsiella quasipneumoniae* strain HKUOPA4	2612	20698	100%	0	100%	

Result of 16S rRNA genes of isolates 36, 37, 39, 40, 43 and 44 (Table 4) and phylogenetic tree of isolates (Figure 4), showed that isolate 36 had similarity with *K. quasipneumoniae* strain TH114 (CP035207.1) with 99.86% percentage identity, isolate 37 had similarity with *K. quasipneumoniae* strain HKUOPL4 (CP014156.1) with 100% percentage identity. However, isolate 39 had similarity with *Enterobacter cloacae* strain Effluent (CP039303.1) with 100% percentage identity, while isolate 40 had similarity with *Enterobacter cloacae* but strain FDAARGOS 1431 (CP077211.1) with 100% percentage identity, and isolate 44 had also similarity with *Enterobacter cloacae* strain NH77 (CP040827.1) with 100% percentage identity. In addition, isolate 43 had similarity with *K. pneumoniae* strain B16KP0198 (CP052520.1) with 100% percentage identity. BLAST-N result of 16S rRNA genes of isolates 52, 56, 59, 60, 62, and 64 (Table 5) and the phylogenetic tree of isolates (Figure 5), showed that isolate 52 had similarity with *K. variicola* strain WCHKP19 (CP028555.2) with 100% percentage identity. Nevertheless, isolate 56 had similarity with *E. roggenkampii* strain 704SK10 (CP022148.1) with 100% percentage identity, but isolate 59 had similarity with *E. asburiae* str. AEB30 (CP046618.1) with 99.91% percentage identity. Moreover, isolates 60 and 64 had similarity with *Enterobacter cloacae* strain FDAARGOS 1431 (CP077211.1) with 100% percentage identity, while isolate 62 had similarity with *K. pneumoniae* strain FDAARGOS 1076 (CP068139.1) with 99.86% percentage identity.

The six new species of cellulolytic bacteria from *Macrotermes gilvus* Hagen gut (Table 6) were identified as *Enterobacter cloacae* (isolates 1, 3, 6, 7, 10, 14, 19, 20, 25, 28, 39, 40, 44, 60, and 64), *K. pneumoniae* (isolates 24, 43, and 62), *K. quasipneumoniae* (isolates 29, 36, and 37), *K. varicolla* (isolate 52), *E. roggenkampii* (isolate 56), and *E. asburiae* (isolate 59).

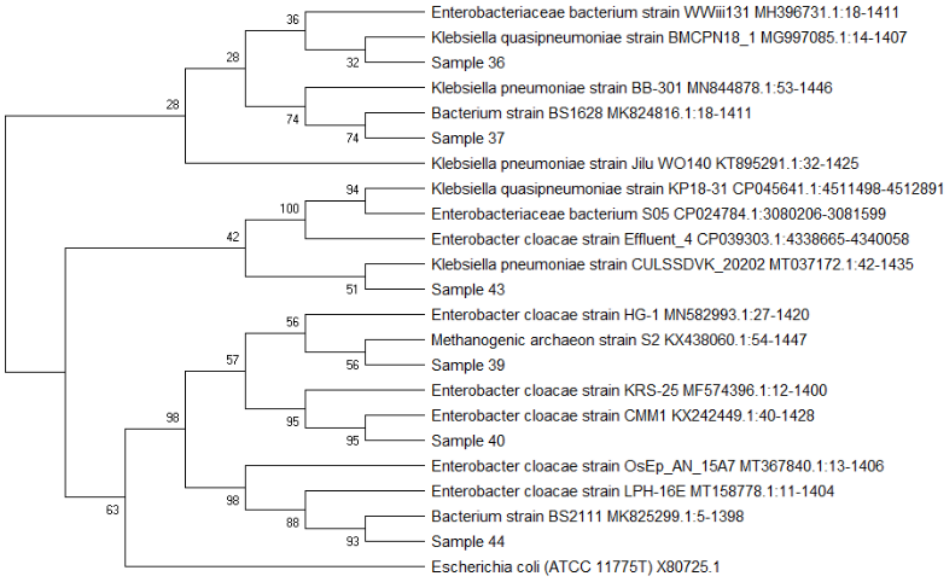

Figure 4. Dendrogram of bacterial isolates 36, 37, 39, 40, 43, and 44.

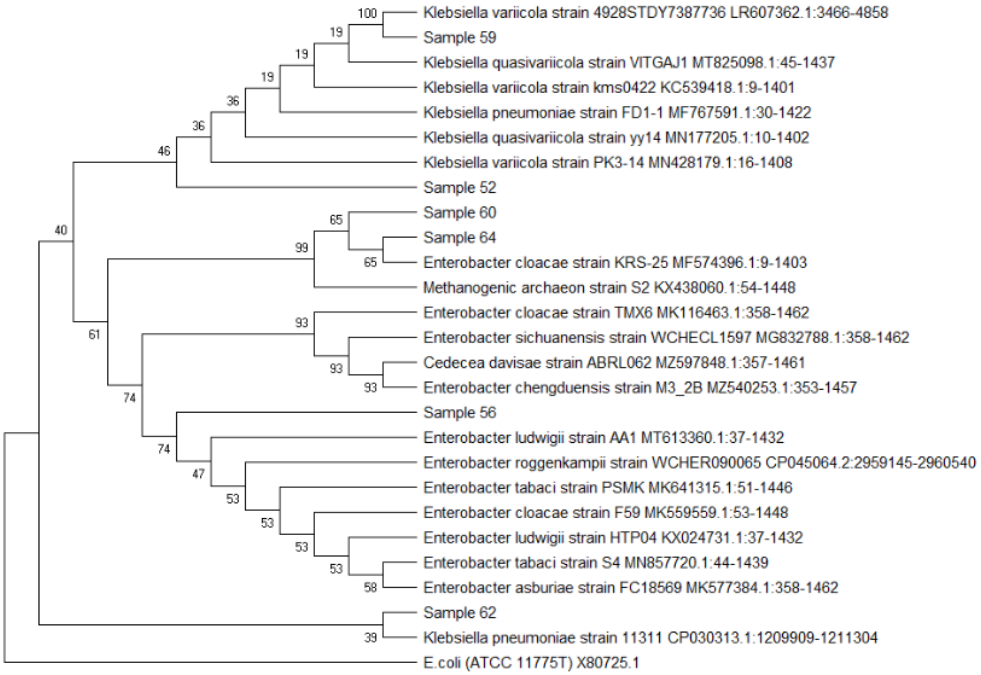

Figure 5. Dendrogram of bacterial isolates 52, 56, 59, 60,62, and 64.

Table 4. BLAST-N of 16S rRNA genes of isolates 36, 37, 39, 40, 43, and 44

Isolates	Accession number	Species	Max score	Total score	Query coverage	E value	Percent. identity	Accession length
36	MH396731.1	*E. bacteriaceae bacterium* strain WWiii131	2575	2575	100%	0	100%	
	MG997085.1	*Klebsiella quasipneumoniae* strain BMCPN18	2569	2569	100%	0	99.93%	
	MN844878.1	*Klebsiella pneumoniae* strain BB-301	2564	2569	100%	0	99.86%	
	CP035207.1	*Klebsiella quasipneumoniae* strain KP18-31	2564	20480	100%	0	99.86%	
	CP035207.1	*Klebsiella quasipneumoniae* strain TH114	2564	20503	100%	0	99.86%	
37	MN844878.1	*Klebsiella pneumoniae* strain BB-301	2575	2575	100%	0	100%	
	CP035207.1	*Klebsiella quasipneumoniae* strain TH114	2575	20480	100%	0	100%	
	MK824816.1	*Bacterium* strain BS1628	2575	2575	100%	0	100%	
	CP014156.1	*Klebsiella quasipneumoniae* strain HKUOPL4	2575	20602	100%	0	100%	
	CP014155.1	*Klebsiella quasipneumoniae* strain HKUOPJ4	2575	20602	100%	0	100%	
39	CP039303.1	*Enterobacter cloacae* strain Effluent	2575	20492	100%	0	100%	
	CP017475.1	*Enterobacter cloacae* strain M12X014451	2575	20480	100%	0	100%	
	CP016906.1	*Enterobacter cloacae* strain isolate SBP-8	2575	17955	100%	0	100%	
40	MF574396.1	*Enterobacter cloacae* strain KRS-25	2566	2566	100%	0	100%	
	KT933255.3	*Enterobacter* sp. MM034	2566	2565	100%	0	100%	
	KX242266.1	*Enterobacter cloacae* strain OS5.8	2566	2565	100%	0	100%	
	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2566	20484	100%	0	100%	
43	CP052520.1	*Klebsiella pneumoniae* strain B16KP0198	2575	20591	100%	0	100%	5309291
	CP052522.1	*Klebsiella pneumoniae* strain B16KP0183	2575	20591	100%	0	100%	5307940
	CP052487.1	*Klebsiella pneumoniae* strain C16KP0024	2575	20502	100%	0	100%	5307337
	CP052324.1	*Klebsiella pneumoniae* strain E16KP0032	2575	20575	100%	0	100%	5149058
	MT037172.1	*Klebsiella pneumoniae* strain CULSSDVK	2575	2575	100%	0	100%	1476
44	CP040827.1	*Enterobacter cloacae* strain NH77	2575	20536	100%	0	100%	
	MK825299.1	*Bacterium* strain BS2111	2575	2575	100%	0	100%	

Table 5. BLAST-N of 16S rRNA genes of isolates 52, 56, 59, 60,62, and 64

Isolates	Accession number	Species	Max score	Total score	Query coverage	E value	Percent. identity
52	CP02855.2	*Klebsiella variicola* strain WCHKP19	2573	20588	100%	0	100%
	LR130543.1	*Klebsiella variicola* strain 04153260899A	2573	20588	100%	0	100%
56	CP045064.2	*Enterobacter roggenkampii* strain WCHER090065	2579	20567	100%	0	100%
	AP019634.1	*Enterobacter* sp. 18A13 DNA	2579	20277	100%	0	100%
	CP014154.1	*Enterobacter cloacae* strain 339389L	2579	20349	100%	0	100%
	CP022148.1	*Enterobacter roggenkampii* strain 704SK10	2579	20399	100%	0	100%
	CP019839.1	*Enterobacter roggenkampii* strain R11	2579	20438	100%	0	100%
59	CP046618.1	*Enterobacter asburiae* str AEB30	2036	16217	100%	0	99.91%
	AP019534.1	*Enterobacter* sp. 18A13 DNA	2036	16156	100%	0	99.91%
	MK577384.1	*Enterobacter asburiae* strain FC18569	2036	16106	100%	0	99.91%
	CP025034.2	*Enterobacter* sp. SGAjr0187	2036	16189	100%	0	99.91%
	CP033800.1	*Enterobacter roggenkampii* strain FDAARGOS 523	2036	16165	100%	0	99.91%
60	MF574396.1	*Enterobacter cloacae* strain KRS-25	2577	2577	100%	0	100%
	CP040827.1	*Enterobacter* sp. MM034	2577	1497	100%	0	100%
	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2577	20573	100%	0	100%
62	CP030313.1	*Klebsiella pneumoniae* strain 11311	2567	20316	100%	0	99.86%
	CP052181.1	*Klebsiella pneumoniae* strain F16KP0037	2567	20360	100%	0	99.79%
	CP052177.1	*Klebsiella pneumoniae* strain F16KP0045	2567	20335	100%	0	99.79%
	LR134217.1	*Klebsiella pneumoniae* strain NCTC10317	2567	20333	100%	0	99.86%
	CP068139.1	*Klebsiella pneumoniae* strain FDAARGOS 1076	2567	22731	100%	0	99.86%
64	MF574396.1	*Enterobacter cloacae* strain KRS-25	2577	2577	100%	0	100%
	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2577	20573	100%	0	100%

Table 6. Summary of BLAST-N of 16S rRNA genes of bacterial isolates

Isolates	Accession number	Species	Max score	Total score	Query coverage	E value	Percent. identity
1	CP046116.1	*Enterobacter cloacae* strain CBG15936	1661	13278	100%	0	100%
3	CP046116.1	*Enterobacter cloacae* strain CBG15936	1530	12230	100%	0	100%
6	MT138539.1	*Enterobacter cloacae* strain MBB8	1507	1507	100%	0	99.88%
7	MT138639.1	*Enterobacter cloacae* strain MBB8	1570	1570	100%	0	100%
10	MN853688.1	*Enterobacter cloacae* strain VITGTJ1	1526	1526	100%	0	99.88%
14	CP040827.1	*Enterobacter cloacae* strain NH77	2597	20713	100%	0	99.86%
19	CP046116.1	*Enterobacter cloacae* strain CBG15936	1807	14446	100%	0	100%
20	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2621	20927	100%	0	100%
24	CP052537.1	*Klebsiella pneumoniae* strain B16KP0141	2601	20770	100%	0	99.86%
25	CP040827.1	*Enterobacter cloacae* strain NH77	2612	20831	100%	0	100%
28	CP040827.1	*Enterobacter cloacae* strain NH77	2606	20787	100%	0	99.93%
29	CP014156.1	*Klebsiella quasipneumoniae* strain HKUOPL4	2612	20898	100%	0	100%
36	CP035207.1	*Klebsiella quasipneumoniae* strain TH114	2564	20503	100%	0	99.86%
37	CP014156.1	*Klebsiella quasipneumoniae* strain HKUOPL4	2575	20602	100%	0	100%
39	CP039303.1	*Klebsiella quasipneumoniae* strain Effluent	2575	20492	100%	0	100%
40	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2566	20484	100%	0	100%
43	CP052520.1	*Klebsiella pneumoniae* strain B16KP0198	2575	20591	100%	0	100%
44	CP040827.1	*Enterobacter cloacae* strain NH77	2575	20536	100%	0	100%
52	CP028555.2	*Klebsiella variicola* strain WCHKP19	2573	20588	100%	0	100%
56	CP045054.2	*Enterobacter roggenkampii* strain WCHER090065	2579	20399	100%	0	100%
59	CP046618.1	*Enterobacter asburiae* str. AEB30	2036	16217	100%	0	99.91%
60	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2577	20573	100%	0	100%
62	CP068139.1	*Klebsiella pneumoniae* strain FDAARGOS 1076	2567	22731	100%	0	99.86%
64	CP077211.1	*Enterobacter cloacae* strain FDAARGOS 1431	2577	20573	100%	0	100%

Discussion

Termites are organisms that play a role in ecosystems, such as decomposing organic matter into nutrients, modifying soil physically and chemically, maintaining the stability of nitrogen and carbon scheme, and raising the activity of other microorganisms (Wright and Jones 2006; Subekti 2012). A total of 3,106 species of termite have been identified and 10% are found in Indonesia, with 5% being pests that are very dangerous for plantations. Termites are widely distributed in tropic and subtropic areas (Arif et al. 2019). Termites consume 50-100% of the biomass from dead plants in the tropics, with 74-99% is cellulose and 65-87% is hemicellulose (Sharma et al., 2015). The *Bacillus subtilis* isolated from low-level termite *Reticulitermes santonensis* has xylanase activity (Tarayre et al. 2013), whereas *B. licheniformis* HI-08 isolated from low-level termite *Heterotermes indocola* produces cellulolytic enzyme (cellulase) (Afzal et al. 2019). Moreover, Ayeronfe et al. (2019) isolated three bacteria from low-level termite *Coptotermes curvignathus* are identified as *Lysinibacillus* sp., *Bacillus* sp., and *Acinetobacter* that produces ligninolytic enzyme (manganese peroxidase, laccase, and lignin peroxidase). Five bacteria, namely *Paenibacillus lactis*, *L. macrolides*, *Stenotrophomonas maltophilia*, *L. fusiformis*, and *B. cereus* are isolated from low-level termite *Psammotermes hypostoma* which shows carboxymethyl cellulase (CMCase) activity (Ali et al. 2019). The *Bacillus* sp. CF96 has β-1,4-glucanase activity isolated from low-level termite *Anacanthotermes* sp (Javaheri-Kermani and Asoodeh 2019). Another *Bacillus* sp. BMP03 from low-level termite *Cryptotermes brevis* has producesd xylanase and carboxymethyl cellulase activity (Tsegaye et al. 2019).

Bacteria *Paenibacillus macerans* IIPSP3 isolated from high-level termite *Termitidae* has produces thermostable xylanase (Dheeran et al. 2012), while *Pseudocitrobacter anthropic* MP-4 from *Microtermes pakistanicus* showed ligninolytic enzyme activity (Li et al. 2019). Three bacteria, namely *Bacillus* sp. B1, *Bacillus* sp. B2, and *Brevibacillus* sp. Br3 that collected from high-level termite *Bulbitermes* sp. have lignocellulolytic enzymes (Kamsani et al. 2016).

Termites have the ability to hydrolyze cellulose with symbiont of bacteria in their digestive tract. The bacteria from high-level *Macrotermes gilvus* gut isolated from Bogor were *Bacillus megaterium* and *Paracoccus yeei* with aerobic and anaerobic conditions had cellulolytic activity (Ferbiyanto et al. 2015). Some species of *Bacillus*, *Cellulomonas*, and *Enterobacter* had also exhibited cellulolytic activities (Sharma et al. 2015). The bacteria that produce amylolytic activity in low-level termite *Coptotermes* sp. gut were *Pseudomonas alcaligenes*, *Brevibacillus parabrevis*, and *Brevibacillus* sp. (Mulyani et al. 2018). Cellulolytic bacteria, such as *Clostridium* sp., member of families Myobacteriaceae, Lactobacillaceae, and Proteus were isolated from termite *Cryptotermes* sp. gut (Peristiwati et al. 2018). *Salmonella paratyphi*, *Shigella flexneri* and *Shigella* sp. were isolated from high-level termite *Macrotermes michaelseni* gut that have cellulolytic activity (Ayitso and Onyango 2016).

Termites are organism with the capability to degrade cellulose into glucose, by a symbiosis bacterial within in their digestive tract. Glucose is a monosaccharide that

makes up the repeating units of cellulose polymers. Glucose is a product of the hydrolysis of cellulose or hemicellulose. Nowadays, hydrolysis of cellulose into glucose has become a concern and is of great very interest to the industrial sector (Peristiwati et al. 2018). Hydrolyzation of cellulose into glucose involves the role of cellulase enzymes followed by fermentation of glucose into ethanol. Glucose as the main raw material enhances bioethanol products by using enzymatic process. Glucose can be obtained from hydrolysis of lignocellulosic biomass, such as wood and plant litter, by using cellulolytic enzymes (Keshk 2016), that obtained from termite gut.

In this research, six novel bacterial species of cellulolytic bacteria isolated from *Macrotermes gilvus* Hagen gut were identified as *Enterobacter cloacae*, *K. pneumoniae*, *K. quasipneumonia*, *K. varicella*, *E. roggenkampii*, and *E. asburiae*. These six bacteria have the ability to hydrolyze cellulose as indicated by their high cellulolytic index on CMC agar media. The six bacteria have the ability use nitrate as the only carbon and energy source. These bacteria can also survive in aerobic and acidic conditions. As reported before, this is a typical characteristic feature of bacteria found in peatlands, which tend to be acidic and rich in oxygen and other organic compounds (Handayani and Aji 2018; Junaedi 2018, Neoh et al. 2017).

The abundance of peatlands, especially in South Sumatra, needs to be studied more deeply, because the biodiversity of potential termite native there. The capability of termite, which have cellulose-degrading capabilities, requires investigation of novel characteristics of cellulolytic enzyme.

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