

Short Communication: Diversity of cellulolytic bacteria isolated from coastal mangrove sediment in Logending Beach, Kebumen, Indonesia

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Abstract. Pramono H, Mariana A, Ryandini D, Sudiana E. 2021. Short Communication: Diversity of cellulolytic bacteria isolated from coastal mangrove sediment in Logending Beach, Kebumen, Indonesia. *Biodiversitas* 22: 1869-1878. Bacteria from mangrove sediments have the potential to produce active enzymes and metabolites needed in the industry. Mangrove ecosystems in various parts of Indonesia have been explored as sources of bacteria. However, the mangrove ecosystems in Logending Beach, Kebumen District, Indonesia are still unexplored. This study aims to determine the diversity of cellulolytic bacteria using the 16S rRNA gene. Ninety-nine bacteria were isolated from Logending coastal mangrove sediments. Most of them (87.87%) had a cellulolytic activity, with the cellulolytic index values ranged from 0.25–13.96. The LG2 isolate had the highest cellulolytic activity (13.96). Molecular characterization of the LG2 isolate based on the 16S rRNA gene showed that it was similar to the *Fictibacillus nanhaiensis* strain JSM 082006. This result is new information on cellulolytic bacteria and might be used as a future source of cellulolytic enzymes.

Keywords: 16S rRNA gene cellulolytic, mangrove, molecular, sediments

INTRODUCTION

Mangrove forests are very productive ecosystems and a potential source of superior bacteria that produce active metabolites and industrial enzymes. In tropical mangroves, 91% of the microbial biomass is bacteria and fungi (Naresh et al. 2019). The existence of microorganisms in mangrove ecosystems is closely related to the stability of the ecosystem. Extracellular enzymes produced by microbes are used to break down complex organic material used by cells as a nutrition source (Thompson et al. 2013). The complexity of the mangrove ecosystems and anthropogenic pressure require microbes to produce extracellular enzymes that function under these complex conditions. Enzymes are susceptible to environmental factors; however, the enzymes produced by microbes in complex ecosystems have a high tolerance to environmental factors (Pal et al. 2018). Mangroves harbor potential bacteria that produce active metabolites and enzymes (Bibi et al. 2017).

The study of microbial interactions with other ecosystem components, such as plant roots, is important for understanding the function and remediation of mangrove ecosystems (Chantarasiri 2015). The bacteria in the mangrove ecosystems break down mangrove leaf litter into organic material used as a source of nutrition by the organisms living in mangrove forests (Glaeser et al. 2013). Environmental factors strongly influence the diversity of bacteria in mangrove ecosystems. Sakhia et al. (2016) showed that bacterial species are affected by mangrove vegetation in the water, especially towards the sea (Liu et al. 2019). Although many microorganisms can degrade

cellulose, only a few produce significant quantities of cell-free bioactive compounds capable of completely hydrolyzing crystalline cellulose in vitro (Rajagopal and Kannan 2015).

Mangrove bacteria can degrade cellulose (Behera et al. 2013; Basak et al. 2016). In a study of mangrove sediments in Dhulibhashani, Sundarbands, India, Basak et al. (2016) found that Proteobacteria, including Bacteroidetes, Acidobacteria, Firmicutes, Actinobacteria, Nitrospirae, Cyanobacteria, Planctomycetes, and Fusobacteria, were dominant using 16S rRNA gene tag sequencing. *Micrococcus*, *Bacillus*, *Pseudomonas*, *Xanthomonas*, and *Brucella* spp. were collected from the Mahanadi River Delta in Odisha, India, which has a high capacity carboxymethyl cellulose hydrolysis (Behera et al. 2013). In Andaman Island mangroves, Rajagopal and Kannan (2017) isolated 19 morphologically distinct Actinobacteria strains from Andaman Island mangroves and 4 strains possessed good cellulose degradation activity, of which strain MHA15 produced the highest cellulase (14,379 IU/mL). Taxonomically, the four strains are members of the genus *Actinoalloteichus* differing from *Actinoalloteichus cyanogriseus* (Behera et al. 2013). Pal et al. (2018) isolated bacteria strains from the Indian Ganges Delta that belongs to the family Bacillaceae and related to the genus *Fictibacillus* based on 16S rRNA sequencing.

Several studies have been carried out on the cellulolytic bacterial diversity of several mangrove ecosystems in Indonesia. Hastuti et al. (2015) found four cellulolytic bacteria species (*Micrococcus luteus*, *Bacillus pumilus*, *Planococcus citreus*, and *Bacillus cereus*) that originated

from mangrove bark on Waai Beach, Ambon Island. Ambeng et al. (2019) isolated 35 cellulolytic bacteria from mangrove sediments in Pangkep, South Sulawesi, belonging to seven genera, i.e., *Bacillus*, *Staphylococcus*, *Vibrio*, *Micrococcus*, *Alteromonas*, *Escherichia*, and *Listeria*. Kurniawan et al. (2017) isolated *Bacillus pumilus*, *Pseudomonas* sp., *Bacillus amyloliquefaciens*, *Bacillus alvei*, and *Bacillus coagulans* from mangrove sediment on Bangka Island, and *Pseudomonas aeruginosa*, which produced the highest cellulase. Nursyiwani et al. (2020) examined 24 isolates of cellulolytic antimicrobial pathogens from mangrove sediment samples from Dumai, Riau and found that 9 isolates could inhibit the development of pathogenic microbes; and 3 of these isolates were similar to *Bacillus toyonensis*.

Maharsiwi et al. (2020) has identified cellulolytic bacteria on mangrove sponges from the Seribu Islands similar to *Pseudomonas luteola* strain NBRC 103146, *Bacillus cereus* strain 24k, *Pseudomonas aeruginosa* strain DSM 50071, *Microbacterium maritipicum* strain DSM 20578, and *Brachybacterium conglomeratum* strain J 1015. Kurniawan et al. (2019) isolated bacteria from the tin-mining region in West Bangka and identified them as *Bacillus amyloliquefaciens*.

Since there has been no study on bacterial diversity in the mangrove area of the southern coast of Java, especially at Logending Beach, Kebumen, we conducted the study to determine the diversity of cellulolytic bacteria mangrove sediments and identified using 16S rRNA sequencing.

MATERIALS AND METHODS

Study site

Sediments were collected at six locations in the mangrove forest in Logending Beach, Ayah Subdistrict, Kebumen District, Central Java Province, Indonesia (7° 42' 28.0" S and 109° 23' 20.1" E). Two locations are at the estuary and three locations along the coast (Figure 1). The samples were processed in the Microbiology Laboratory of the Faculty of Biology, Jenderal Soedirman University, Purwokerto, Banyumas, Indonesia.

Isolation of mangrove sediment bacteria

Bacteria were isolated using the pour plate technique on nutrient agar (NA) medium. Ten g of sediment samples were homogenized in 90 mL physiological saline and then diluted up to 10^{-5} fold. The last three dilutions were cultured in NA medium and incubated for 24 hours at room temperature. Bacterial colonies grow on the diluted medium of 10^{-3} , 10^{-4} , and 10^{-5} were counted, and the bacterial density was reported as the total number of bacteria per gram of mangrove sediment. Bacterial diversity was observed from colony morphology. The colonies were further purified by the quadrant streak technique to isolate and characterize bacterial isolates.

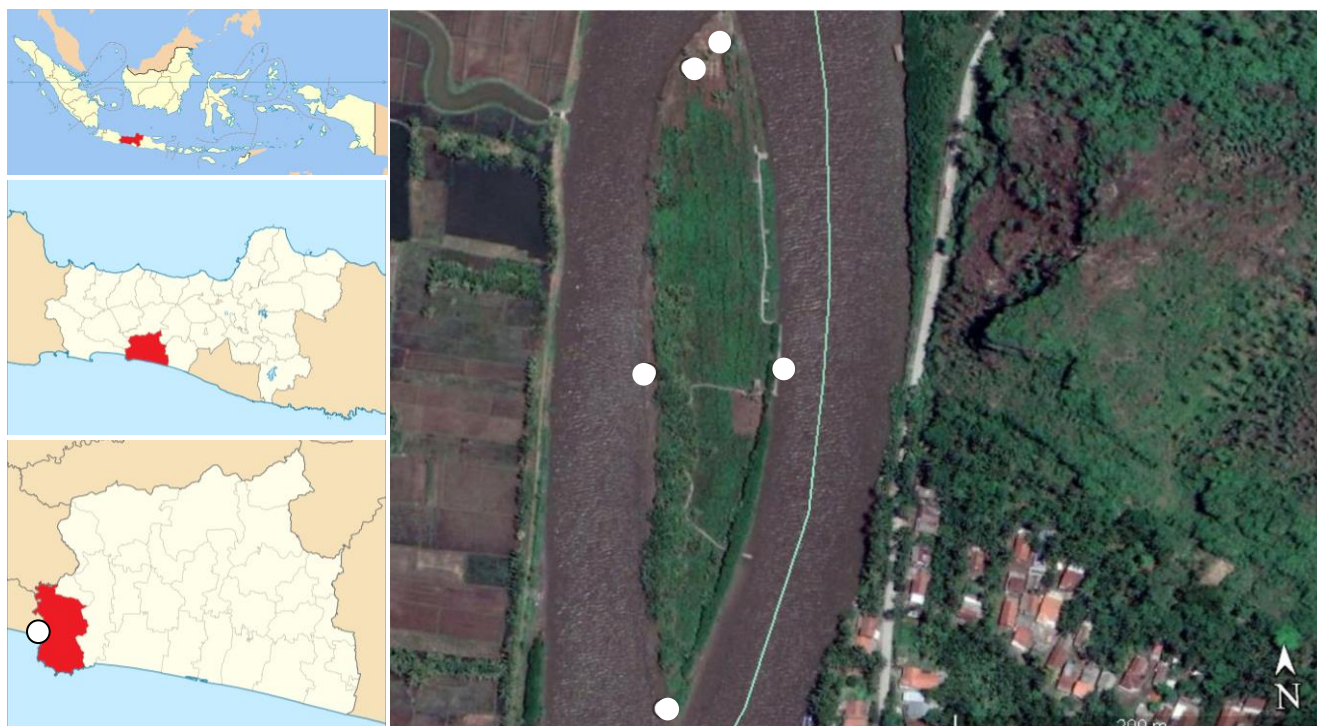


Figure 1. Sampling locations (white circles) in the mangrove forest of Logending Beach, Ayah Subdistrict, Kebumen District, Central Java Province, Indonesia

Cellulolytic activity test

Bacterial isolates were inoculated on CMC agar and incubated for 24 hours. The cellulolytic activity was observed by dripping Congo Red solution on the culture surface. The formation of clear zones around a colony indicates cellulolytic activity. The clear zone and colony diameters were measured, and enzymatic activity was calculated by dividing the diameter of the clear zone by the colony diameter.

Isolation of bacterial genomic DNA

Bacterial chromosomal DNA was isolated using the method of Klijn et al. (1991) with some modifications. A single bacterial colony was suspended in 50 μ L 10 mM Tris-HCl (pH 8) buffer solution containing 400 μ g lysozyme and incubated for 1 hour at 37°C. Cell lysis was improved by adding 50 μ L 10% SDS and 250 μ L buffer. The mixture was centrifuged at 5,000 \times g for 10 minutes. The supernatant was transferred to a new tube, and 60 mL 3 M sodium acetate and 1 mL 96% chilled ethanol were added. After centrifuging at 12,000 \times g for 15 minutes, the DNA pellet was placed in 300 μ L 70% ethanol and centrifuged at 8,000 \times g for 5 minutes. The pellet was dried at room temperature until all the ethanol evaporated, and then it was dissolved in 50 μ L TE buffer. The chromosomal DNA was analyzed by agarose gel electrophoresis.

16S rRNA gene amplification and phylogenetic analysis

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using a DNA chromosome template. The PCR mixture consisted of 8.55 μ L ddH₂O, 3 μ L 10 \times PCR buffer, 0.45 μ L 5 mM dNTP, 1.05 μ L 0.6 mM MgCl₂, 2 μ L primers, 0.15 μ L *Taq* polymerase, and 0.6 μ L chromosomal DNA. PCR analysis was performed on a thermocycler, with an initial denaturation step at 94°C for 4 min, followed by 34 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 2 min. The primers used to amplify the 16S rRNA gene were Bact27F (5'-AGAGTTTGATCATG GCTCAG-3') and Uni1492R (5'-GGTTACCTTGT TACGACTT-3'). The PCR products were verified by agarose gel electrophoresis and then sequenced.

Data analysis

Using a BLAST search, selected 16S rRNA sequences were compared to other sequences in GenBank to determine the level of similarity. Then, a phylogenetic tree of the 16S rRNA sequences was constructed using MEGA ver. 6. (Tamura et al. 2013)

RESULTS AND DISCUSSION

Diversity of cellulolytic bacteria in coastal mangrove sediment from Logending

In total, there were 99 bacteria strains isolated from the Logending mangrove sediments (Table 1). The highest number of isolates was obtained from the first sampling location (58 isolates), followed by the 3rd and 5th sampling locations (53 isolates from each location), and the lowest

number was obtained from the 6th sampling location (14 isolates) (Figure 2). The first sampling point is directly opposite the river flow direction, while the 6th sampling location faces the sea. The difference in the number of isolates among sampling points is probably due to the sediment type. The result showed that 87 bacterial isolates have cellulolytic activity, as indicated by a clear zone around the colony on CMC agar containing Congo Red (Figure 3).

The cellulolytic index (CI) reflects the ability of the cellulase produced by bacterial isolates to degrade cellulose on CMC agar. The CI values ranged from 0.25 to 13.96 (Table 1), and 12 isolates had a CI > 3.0. The highest CI (13.96) was produced by the LG2 isolate (Table 1).

Mangrove sediments in Logending Beach have a high potential for cellulolytic bacteria, and only a few isolates have lower CI values than in other mangrove areas in Indonesia. Kurniawan et al. (2017) only found five cellulolytic bacteria from mangrove sediments at Sungailiat and Tukak Sadai on Bangka Island. Nursyirwani et al. (2020) obtained 24 cellulolytic bacterial isolates with CI values ranging from 1.00 to 2.86.

Many cellulolytic bacteria have been isolated from sediments and tropical mangrove soils. Functional metagenomic analyses have shown that mangrove land has high cellulolytic enzyme activity. Specific sequences involved in cellulose degradation were identified in mangroves from Brazil (Thompson et al. 2013). High cellulolytic activity can be found in sediments with high mangrove litter levels, which creates a cellulose-rich environment for bacterial populations.

Identification of 16S rRNA genes

Based on 16S rRNA sequences and using BLAST from NCBI, it showed the LG2 isolate had the highest similarity (99.86%) to *Fictibacillus nanhaiensis* strain JSM 082006 in GenBank (Table 2). Other closely related strains were *F. halophilus* (99.79%), *F. phosphorivorans* (99.86%), *F. barbaricus* (98.21%), *F. aquaticus* (98.21%), *F. arsenicus* (97.43.0%), *F. rigui* (97%), *F. enclensis* (96.64%), *F. solisalsi* (96.43%), and *Bacillus tianmuensis* (96.22%) (Table 2).

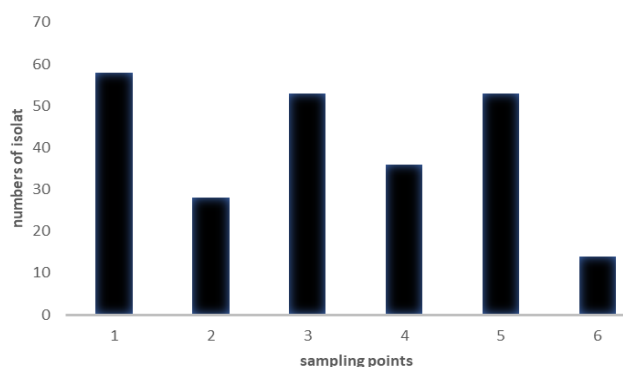


Figure 2. Number of isolates in each sampling locations

Table 1. Isolat richness and densities (means + stdev)

Isolate name	Sampling points (n = 6)					
	1	2	3	4	5	6
LG 1	0	0	0	0	2500,00 ± 577,35	0
LG 2	633,33 ± 115,47	25125,00 ± 3544,36	3000,00 ± 433,01	338333,30 ± 17559,42	17833,33 ± 2565,80	16833,33 ± 288,68
LG 3	0	0	0	2166,67 ± 577,35	5000,00 ± 500,00	0
LG 4	350000,00 ± 28867,51	1250,00 ± 204,12	16666,67 ± 1443,38	0	453333,30 ± 45092,50	500,00 ± 86,60
LG 5	0	0	0	0	5000,00 ± 500,00	0
LG 6	0	250000,00 ± 40824,83	33333,33 ± 5773,50	4166,67 ± 577,35	18333,33 ± 2886,75	16833,33 ± 288,68
LG 7	40000,00 ± 8231,04	19969125,00 ± 1353518,77	58333,33 ± 5773,50	183333,30 ± 28867,51	54000,00 ± 3605,55	16666,67 ± 2886,75
LG 8	0	0	0	0	1666,67 ± 288,68	0
LG 9	0	0	166,67 ± 14,43	0	0	0
LG 10	0	0	333,33 ± 43,30	0	16666,67 ± 2886,75	0
LG 11	0	0	2333,33 ± 144,34	0	35000,00 ± 5000,00	0
LG 12	0	0	17500,00 ± 1322,88	0	0	0
LG 13	13333,33 ± 2886,75	62500,00 ± 5000,00	8333,33 ± 2886,75	5000,00 ± 288,68	18333,33 ± 2886,75	0
LG 14	1666,67 ± 288,68	50375,00 ± 3685,56	500,00 ± 43,30	500,00 ± 50,00	17833,33 ± 2565,80	0
LG 15	0	0	0	0	19000,00 ± 1732,05	0
LG 16	0	125000,00 ± 28867,51	0	0	1666,67 ± 288,68	0
LG 17	46166,67 ± 5392,90	3000,00 ± 408,25	103333,30 ± 2886,75	15000,00 ± 2500,00	8500,00 ± 866,03	334000,00 ± 27712,81
LG 18	0	0	0	0	3333,33 ± 288,68	0
LG 19	0	125000,00 ± 41231,06	0	0	173333,30 ± 46188,02	0
LG 20	0	0	0	0	1666,67 ± 288,68	0
LG 21	0	0	666,67 ± 57,74	1666,67 ± 288,68	0	0
LG 22	0	0	0	11666,67 ± 2886,75	16666,67 ± 2886,75	0
LG 23	0	0	0	3333,33 ± 288,68	0	0
LG 24	0	0	0	7666,67 ± 577,35	0	0
LG 25	1328333,00 ± 183462,08	149625,00 ± 33623,84	38333,33 ± 7637,63	1666,67 ± 288,68	9333,33 ± 2081,67	17666,67 ± 3511,88
LG 26	0	0	1666,67 ± 152,75	166666,70 ± 28867,51	0	0
LG 27	1666,67 ± 288,68	0	0	0	0	0
LG 28	1666,67 ± 288,68	0	0	0	0	0
LG 29	5000,00 ± 0,00	0	0	15000,00 ± 2000,00	0	0
LG 30	0	0	0	0	1666,67 ± 288,68	0
LG 31	0	0	0	3333,33 ± 288,68	3500,00 ± 500,00	0
LG 32	3333,33 ± 577,35	125,00 ± 20,41	0	0	0	0
LG 33	500,00 ± 86,60	0	0	0	1666,67 ± 288,68	0
LG 34	1666,67 ± 288,68	0	0	0	0	0
LG 35	18333,33 ± 2886,75	0	16666,67 ± 5773,50	166,67 ± 28,87	25000,00 ± 2886,75	0
LG 36	6666,67 ± 288,68	0	0	0	0	0
LG 37	1833,33 ± 144,34	0	0	0	0	0

LG 38	6666,67 ± 288,68	0	0	0	0	0
LG 39	3333,33 ± 577,35	0	0	0	0	0
LG 40	1666,67 ± 381,88	0	0	0	0	0
LG 41	333,33 ± 57,74	0	0	0	0	0
LG 42	16833,33 ± 2753,79	0	0	0	16666,67 ± 2886,75	0
LG 43	166,67 ± 28,87	0	0	0	500,00 ± 50,00	0
LG 44	3500,00 ± 288,68	0	0	0	0	0
LG 45	333,33 ± 57,74	0	0	0	0	0
LG 46	2500,00 ± 500,00	0	7500,00 ± 866,03	8333,33 ± 1154,70	166833,30 ± 28724,26	0
LG 47	0	0	0	0	333,33 ± 28,87	0
LG 48	16666,67 ± 2886,75	0	5000,00 ± 866,03	0	3833,33 ± 763,76	0
LG 49	0	1250,00 ± 288,68	0	0	166,67 ± 28,87	0
LG 50	0	0	9000,00 ± 1322,88	2666,67 ± 288,68	1666,67 ± 288,68	0
LG 51	0	0	0	0	33666,67 ± 2309,40	0
LG 52	0	0	0	0	166,67 ± 28,87	0
LG 53	13333,33 ± 1732,05	0	0	833,33 ± 144,34	0	0
LG 54	0	0	1666,67 ± 288,68	0	0	0
LG 55	15000,00 ± 866,03	0	40000,00 ± 1000,00	16666,67 ± 2886,75	1000,00 ± 100,00	166,67 ± 28,87
LG 56	0	0	1666,67 ± 288,68	0	0	0
LG 57	0	0	3333,33 ± 288,68	0	0	0
LG 58	0	0	1666,67 ± 288,68	0	0	0
LG 59	0	0	1666,67 ± 288,68	0	0	0
LG 60	666,67 ± 57,74	100000,00 ± 20412,41	0	167000,00 ± 28583,21	0	0
LG 61	0	110000,00 ± 8660,25	0	0	2666,67 ± 288,68	0
LG 62	666,67 ± 57,74	0	500,00 ± 50,00	0	0	0
LG 63	666,67 ± 57,74	0	0	0	0	0
LG 64	166,67 ± 28,87	0	0	0	0	0
LG 65	333,33 ± 57,74	0	0	0	0	0
LG 66	166,67 ± 28,87	0	0	0	0	0
LG 67	0	0	0	166,67 ± 28,87	0	0
LG 68	0	662500,00 ± 75000,00	8666,67 ± 1527,53	1666,67 ± 763,76	0	0
LG 69	0	0	0	0	1666,67 ± 288,68	0
LG 70	0	0	0	0	5000,00 ± 500,00	0
LG 71	0	0	0	0	3333,33 ± 288,68	0
LG 72	0	0	0	0	1666,67 ± 288,68	0
LG 73	0	0	0	1666,67 ± 288,68	0	0
LG 74	0	0	0	3333,33 ± 288,68	0	0
LG 75	0	0	0	6666,67 ± 577,35	0	0
LG 76	0	0	0	15000,00 ± 1000,00	0	0
LG 77	0	0	0	333,33 ± 28,87	0	0
LG 78	0	0	50000,00 ± 5000,00	666,67 ± 144,34	0	0
LG 79	0	0	0	333,33 ± 28,87	0	0
LG 80	0	0	0	6666,67 ± 577,35	50000,00 ± 5000,00	0
LG 81	133333,30 ± 14433,76	0	3333,33 ± 763,76	0	13166,67 ± 1154,70	166,67 ± 28,87
LG 82	0	0	4500,00 ± 866,03	0	1166,67 ± 288,68	0

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LG 83	0	0	333,33 ± 57,74	0	166,67 ± 72,17	0
LG 84	0	0	0	0	166,67 ± 28,87	0
LG 85	0	0	166,67 ± 28,87	0	0	0
LG 86	0	0	166,67 ± 28,87	0	0	0
LG 87	0	0	9833,33 ± 288,68	0	0	0
LG 88	0	0	166,67 ± 28,87	0	0	0
LG 89	0	0	166,67 ± 28,87	0	0	0
LG 90	0	0	333,33 ± 57,74	0	0	0
LG 91	0	375,00 ± 50,00	0	0	0	0
LG 92	2000,00 ± 433,01	0	0	0	0	0
LG 93	1500,00 ± 346,41	0	166666,70 ± 28867,51	0	0	0
LG 94	3333,33 ± 288,68	0	0	0	166,67 ± 28,87	0
LG 95	0	0	0	0	3333,33 ± 288,68	0
LG 96	0	0	0	0	28333,33 ± 7637,63	0
LG 97	0	0	0	500,00 ± 50,00	0	0
LG 98	0	0	500,00 ± 50,00	0	0	0
LG 99	0	0	1166,67 ± 288,68	0	0	0
LG 100	0	0	166,67 ± 28,87	0	0	0
LG 101	5000,00 ± 866,03	0	0	0	0	0
LG 102	5000,00 ± 866,03	0	0	0	0	0
LG 103	16666,67 ± 2886,75	875,00 ± 150,00	17166,67 ± 2565,80	0	0	0
LG 104	0	0	500,00 ± 50,00	0	0	0
LG 105	0	0	1166,67 ± 288,68	0	0	0
LG 106	0	0	166,67 ± 28,87	0	0	0
LG 107	0	0	0	3333,33 ± 288,68	0	0
LG 108	33333,33 ± 2886,75	0	0	0	0	0
LG 109	16666,67 ± 2886,75	0	0	0	0	0
LG 110	16666,67 ± 2886,75	0	0	0	0	0
LG 111	16666,67 ± 2886,75	0	0	0	0	0
LG 112	166666,70 ± 158771,32	0	0	0	0	0
LG 113	0	0	0	0	25000,00 ± 2886,75	0
LG 114	0	12500,00 ± 2516,61	16666,67 ± 2886,75	16666,67 ± 2886,75	0	0
LG 117	0	0	0	0	0	166,67 ± 28,87
LG 118	0	150000,00 ± 8164,97	0	0	0	0
LG 119	16666,67 ± 2886,75	0	0	3666,67 ± 288,68	0	0
LG 120	0	1250,00 ± 300,00	0	0	0	0
LG 121	0	0	0	0	0	333333,30 ± 28867,51
LG 122	0	125,00 ± 33,67	16666,67 ± 2886,75	0	0	0
LG 123	0	0	0	0	0	16666,67 ± 2886,75
LG 126	0	0	16666,67 ± 2886,75	0	166666,70 ± 28867,51	166666,70 ± 28867,51
LG 125	0	250,00 ± 40,82	0	0	0	0
LG 127	0	125,00 ± 45,64	0	0	0	0
LG 128	0	1250,00 ± 191,49	0	0	0	0

LG 129	0	0	16666,67 ± 2886,75	0	166666,70 ± 28867,51	0
LG 130	0	0	166666,70 ± 28867,51	0	0	0
LG 131	0	0	166666,70 ± 28867,51	0	0	0
LG 134	0	0	166,67 ± 28,87	0	0	0
LG 135	33333,33 ± 31754,26	0	0	0	0	0
LG 136	0	0	0	0	166666,70 ± 28867,51	0
LG 137	50000,00 ± 8660,25	0	0	0	0	0
LG 138	0	0	0	0	333333,30 ± 28867,51	0
LG 139	0	0	0	0	166666,70 ± 28867,51	0
LG 140	0	1250,00 ± 191,49	0	0	0	0
LG 141	0	2500,00 ± 95,74	0	0	0	0
LG 142	0	500,00 ± 81,65	0	0	0	0
LG 143	0	0	0	166,67 ± 28,87	0	0
LG 144	0	0	0	0	0	333,33 ± 28,87
LG 145	0	0	0	0	0	166,67 ± 28,87

Table 2. The cellulolytic index of bacterial isolates collected from Logending coastal mangrove sediment

Isolate code	Cellulolytic index
LG2	13.96
LG3	10.60
LG7	6.74
LG19	6.35
LG18	4.83
LG14	4.08
LG73	3.91
LG115	3.80
LG139	3.64
LG20	3.42
LG13	3.21
LG16	3.07

In the phylogenetic tree, LG2 was closely related to the *Fictibacillus* group (Figure 4), suggesting that they have a common ancestor and share some features and functionality (Table 2, Figure 4). The genus *Fictibacillus* is Gram-positive, motile, rod-shaped, endospore-forming bacteria (Pal et al. 2018); however, studies on their cellulolytic activity are still limited. A study by Chen et al. (2020) showed that *Fictibacillus* sp. YS-26 isolated from soft corals in Yongxing Island, Hainan Province, China, have high cellulolytic activity and can degrade

lignocellulose. The cellulolytic activity of *Fictibacillus* from mangrove sediments has never been reported. Therefore, the results of this study are considered as new information on the cellulolytic activity of *Fictibacillus* from mangroves.

Bacterial species that adapt to mangrove ecosystems are a potential source for biotechnology. Mangroves are a rich resource for discovering new bacterial and fungal species that produce enzymes and molecules to be used in human life, i.e., agriculture, industry, and bioremediation (Dias et al. 2009; Dourado et al. 2012). The ability to convert lignocellulosic substrates into simple nutrients is crucial for the carbon cycle and microbial survival, especially in high substrates environments. Lignocellulosic materials are very difficult to degrade due to their dense, compact structures, which protect plants against microbial attack (Kumar et al. 2008). Microorganisms that can degrade plant biomass might only be found in specific environments, such as mangrove ecosystems, where the biomass is abundant.

In conclusion, bacteria with cellulolytic activity were obtained from Logending mangrove sediments. Based on the phylogenetic analysis, isolate LG2 is similar to the *Fictibacillus* group. This study provides new information on the cellulolytic activity of *Fictibacillus* from mangroves. It can produce enzymes and chemicals that can be beneficial for human life, such as agriculture, industry, and bioremediation.

Table 2. The similarity of LG2 isolate to other related strains using BLAST-N

Description	Max score	Total score	Query cover	E value	Per. Ident	Accession
<i>Fictibacillus nanhaiensis</i> strain JSM 082006 16S ribosomal RNA, partial sequence	2571	2571	99%	0.0	99.86%	NR_117524.1
<i>Fictibacillus halophilus</i> strain AS8 16S ribosomal RNA, partial sequence	2567	2567	100%	0.0	99.79%	NR_149289.1
<i>Fictibacillus phosphorivorans</i> strain Ca7 16S ribosomal RNA, partial sequence	2567	2567	99%	0.0	99.86%	NR_118455.1
<i>Fictibacillus barbaricus</i> strain V2-BIII-A2 16S ribosomal RNA, partial sequence	2484	2484	99%	0.0	98.85%	NR_028967.1
<i>Fictibacillus aquaticus</i> strain GDSW-R2A3 16S ribosomal RNA, partial sequence	2446	2446	100%	0.0	98.21%	NR_159291.1
<i>Fictibacillus arsenicus</i> strain Con a/3 16S ribosomal RNA, partial sequence	2386	2386	100%	0.0	97.43%	NR_042217.1
<i>Fictibacillus rigui</i> strain WPCB074 16S ribosomal RNA, partial sequence	2350	2350	100%	0.0	97.00%	NR_116518.1
<i>Fictibacillus enclensis</i> strain NIO-1003 16S ribosomal RNA, partial sequence	2320	2320	99%	0.0	96.64%	NR_133744.1
<i>Fictibacillus solisalsi</i> strain YC1 16S ribosomal RNA, partial sequence	2300	2300	99%	0.0	96.43%	NR_044387.1
<i>Bacillus tianmuensis</i> strain B5 16S ribosomal RNA, partial sequence	2290	2290	100%	0.0	96.22%	NR_116701.1

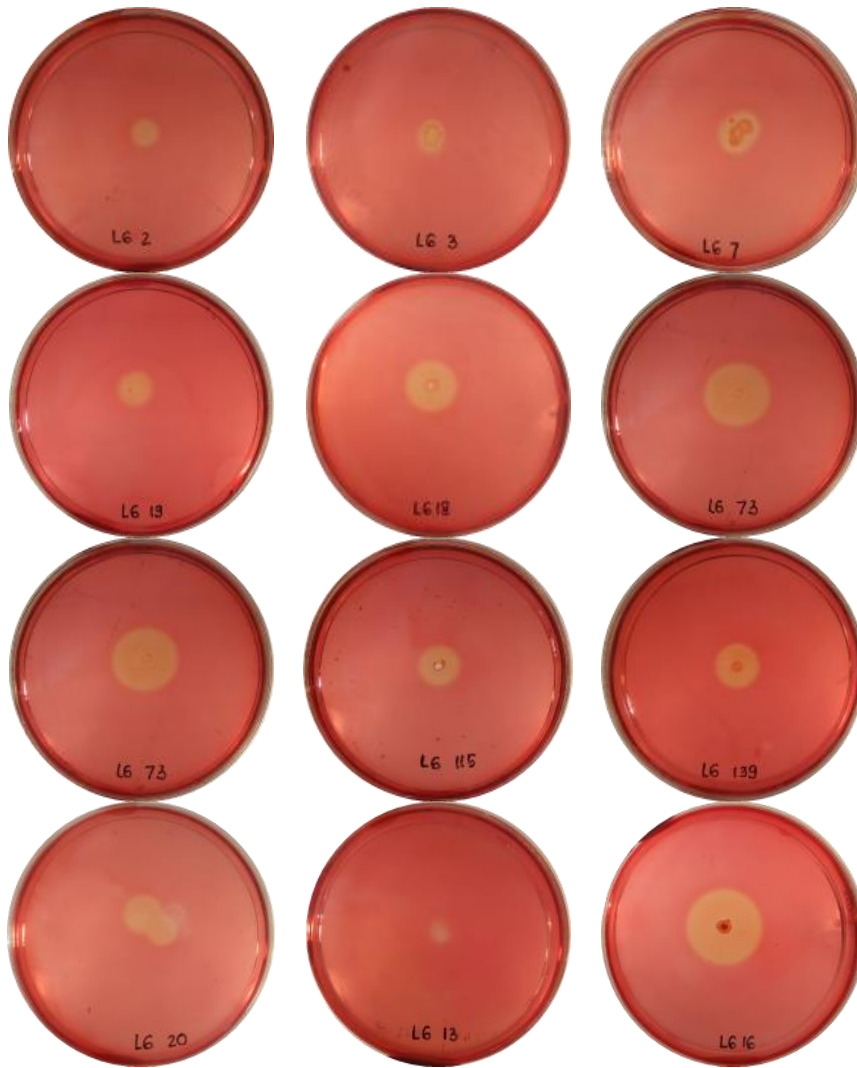


Figure 3. Cellulolytic activity of some bacterial isolates collected from Logending mangrove sediments

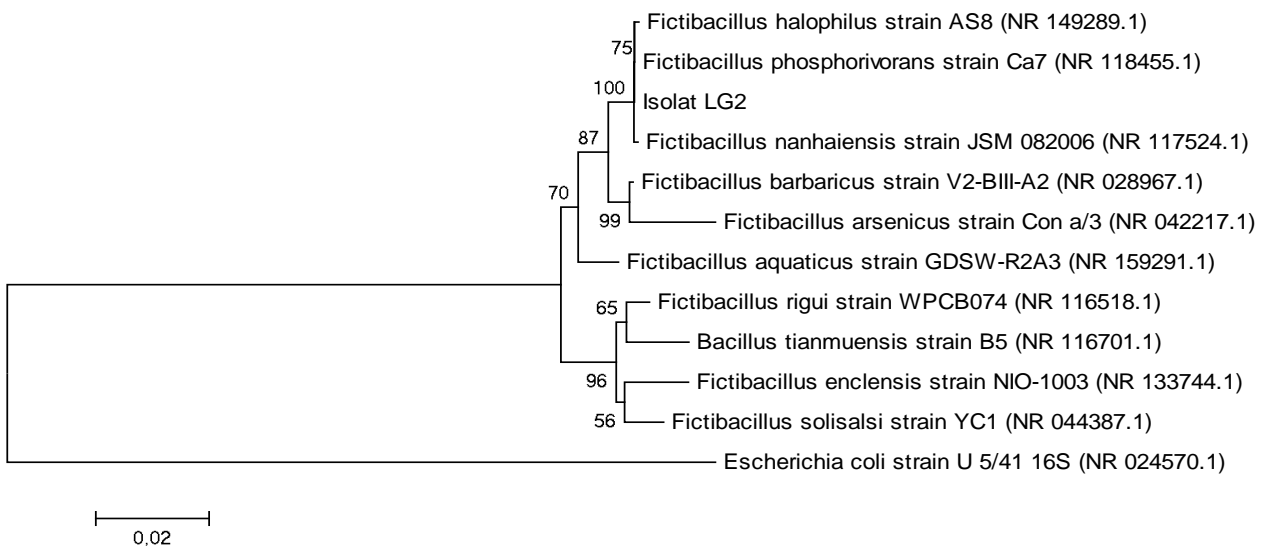


Figure 4. Phylogenetic tree of isolate LG2 and its closely related strains based on 16S rRNA gene sequence in the GenBank database

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