Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati, Vol. 10(2): 204-212, Juni 2025
p-ISSN 2527-3221, e-ISSN 2527-323X, https://ojs.uajy.ac.id/index.php/biota/issue/view/499
DOI: 10.24002/biota.v10i2.11262

Isolation and Screening of Cellulolytic Soil Bacteria from Dramaga Forest in Bogor

Ratu Weridyaningrum[1*], Witiyasti Imaningsih[1], Gusti Nur Aida Fasha[1]

[1]Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat
Jalan Ahmad Yani KM. 36, Loktabat Selatan, Banjarbaru 70714, South Kalimantan, Indonesia
*ratu.weridya@ulm.ac.id *Corresponding Author*

Abstract

Cellulolytic bacteria can produce cellulase enzymes, which allow them to degrade cellulose into simpler chemicals while using it as a growth substrate. These bacteria have drawn a lot of interest due to their unique properties and potential uses, such as in agriculture and the food industry. Soil is one of the habitats of cellulolytic bacteria. This study aims to determine the potential and screening of cellulolytic bacterial isolates. Cellulolytic bacteria were isolated from soil samples and cultured using selective Carboxyl Methyl Cellulose (CMC) media. Five purified isolates were collected, and their cellulase enzyme activity was qualitatively assessed using the Congo red test. Isolated bacteria code Area Jalan Setapak (AJS1) produced a higher clear zone index and will be used for further testing. Quantitative analysis of isolate AJS1 revealed its highest enzyme activity after ten minutes of incubation, with 0.0049 U/mL unit activity and 0.018 U/mg specific activity. Based on morphological and biochemical characteristics, isolated AJS1 was identified as *Corynebacterium* spp.

Keywords: ***Carboxyl methyl cellulose, cellulolytic bacteria, cellulose, cellulase enzyme, soil***

Submitted : 23 March 2025 ; Revised : 20 June 2025 ; Accepted : 20 June 2025

Introduction

Cellulose is one of the most abundant biopolymers on earth, and its breakdown into glucose or oligosaccharides has great potential in various industrial applications (Yunus & Kuddus, 2021). Nonetheless, cellulose possesses certain inherent limitations, such as limited resistance to wrinkling, low solubility in solvents, and the absence of thermoplastic behavior. To enhance its functional properties, it is essential to apply controlled chemical or physical surface modifications, including the breakdown of its structure (Benalaya *et al.*, 2024). Furthermore, the breakdown of cellulose involves an enzyme called cellulase. Cellulase enzymes break the β-1,4 bonds in the cellulose chain, thus helping cellulose degradation. Cellulases play a crucial role in the biodegradation of cellulose present in organic waste and have been applied across multiple sectors. In the healthcare field, they serve as an alternative to antibiotics by targeting *Pseudomonas* biofilms (Kamali *et al.*, 2021). In the food and beverage industry, cellulases are utilized in fruit juice production and in enhancing flavors and fragrances (Ejaz *et al.*, 2021). The textile industry employs these enzymes in bio-washing and bleaching processes, while the paper industry uses the enzymes for deinking applications (Monika & Rukhaya, 2025). In the field of biotechnology, cellulases contribute to bioethanol production (Ranganathan *et al.*, 2022). Meanwhile, in the manufacturing sector, they are incorporated into formulations for detergents as well as cleaning and laundry products (Abdella *et al.*, 2024). Therefore, the exploration of cellulase-producing organisms, particularly microorganisms, is essential for advancing their development and utilization across a wide range of industrial sectors.

Microorganisms capable of degrading cellulose produce three main types of cellulase enzymes, namely exoglucanases (EC 3.2.1.74), endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) (Borthakur *et al.*, 2024). Cellulase enzymes are produced by various types of microorganisms, including

Copyright© 2025. Ratu Weridyaningrum, Witiyasti Imaningsih, Gusti Nur Aida Fasha

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License

How to Cite : Weridyaningrum, R., Imaningsih, W., & Fasha, G. N. A. (2025). Isolation and Screening of Cellulolytic Soil Bacteria from Dramaga Forest in Bogor. Jurnal Ilmiah Ilmu-Ilmu Hayati 10(2):204-212.

bacteria (Wang *et al.*, 2024), yeast (Fu *et al.*, 2022), and actinomycetes (Putri & Setiawan, 2019). Among these microorganisms, cellulolytic bacteria produce cellulase enzymes efficiently and adaptively to various environmental conditions. Cellulolytic bacteria are commonly found in environments rich in organic matter, such as forest soils (Bautista-Cruz *et al.*, 2024) and agricultural waste (Akhtar & Goyal, 2015). Cellulolytic bacteria from soil play an important role in the carbon cycle in nature as they help break down cellulose-containing organic matter, such as plant debris, into compounds that can be utilized by other organisms (Gatpatan *et al.*, 2024). Soil is a rich source of cellulolytic bacteria because it contains various organic materials derived from plant decomposition. Some soil bacteria that have been known to produce cellulase come from the genera *Aeromonas, Bacillus, Chryseobacterium, Lysinibacillus, Pseudomonas,* and *Vibrio* (Bamrungpanichtavorn *et al.*, 2023).

In addition to their role in nutrient cycling in the ecosystem, these bacteria also have potential biotechnological applications. For example, cellulase enzymes produced by cellulolytic bacteria can be used in the biofuel industry to convert cellulosic biomass into bioethanol, a renewable energy source (Z. Fu *et al.*, 2024). Cellulolytic bacteria are also used in the textile, paper, detergent, agriculture, food, and animal feed industries (Rahman *et al.*, 2021). With these various benefits, research on cellulolytic bacteria from soil continues to develop to explore their potential and application in various fields, especially in efforts to ensure the sustainability and efficiency of organic matter processing. Given the broad spectrum of applications and ecological significance of cellulolytic bacteria, particularly those derived from soil, further research into their diversity, enzymatic activity, and industrial prospects is essential. Exploring these microorganisms not only contributes to the understanding of microbial ecology and the natural carbon cycle but also offers promising opportunities for sustainable biotechnological innovations. Therefore, this study aims to isolate, identify, and characterize cellulolytic bacteria from soil environments, with the goal of assessing their cellulase-producing capabilities and evaluating the potential applications.

Research Method

Material

The materials used are soil samples, Carboxy Methyl Cellulose (CMC) media, Nutrient Agar (NA) media, 0.85% physiological salt solution (NaCl), safranin solution, lugol-iodine solution, malachite green solution, 0.2 M NaCl solution, Congo red reagent 0.1%, phosphate buffer 0. 05 M pH 7, glucose stock solution, bovine serum albumin (BSA) stock solution, dinitrosalicylic acid (DNS) reagent, Bradford reagent, acid-fast stain reagents, 3% H_2O_2 solution, 1% starch, and distilled water.

Sample Collection

Soil samples were collected from the forest area in Dramaga, Bogor. A total of 10 grams of soil samples were taken at a depth of approximately 10 cm below the soil surface. Soil samples were wrapped in aluminum foil, placed in sample plastic, and then stored at room temperature.

Isolation and Selection of Cellulolytic Bacteria

Bacterial isolation was carried out using the dilution method. A total of 1 gram of soil sample was dissolved in 9 mL of 0.85% physiological saline solution (NaCl), and serial dilutions of 10^{-5} to 10^{-7} were made. 0.1 mL samples from the last two dilutions were inoculated into a petri dish containing Carboxy Methyl Cellulose (CMC) media and incubated at 26°C for 24-48 hours. Colonies with different morphology were purified to obtain pure isolates.

Bacterial morphological identification

Bacterial morphological colonies were macroscopically identified by observing their shape, size, surface, optical characterization, color, elevation, edges, and texture. Meanwhile, bacterial cell morphology was characterized using a Gram-staining assay as described by Moyes *et al.* (2009). If the bacteria obtained are Gram-positive bacteria, endospore staining will be performed. The Schaeffer-Fulton method uses a malachite green stain to perform endospore staining on gram-positive bacterial isolates.

Qualitative Test of Cellulolytic Activity

Bacterial isolates were inoculated onto CMC medium and incubated at 26°C for 48 hours. The growing bacterial isolates were stained with Congo red for 15 minutes. Then, the excess Congo red was discarded and washed with 0.2 M NaCl solution (Teather & Wood, 1982). The clear zone formed was observed and measured with the following formula.

$$\text{Clear zone index (mm)}: \frac{\text{Clear zone diameter (mm)} - \text{Bacterial colony diameter (mm)}}{\text{Colony diameter (mm)}}$$

The bacterial isolate with the highest cellulolytic index was used for further testing.

Quantitative Test of Cellulase Enzyme Activity

Potential bacterial isolate was inoculated into liquid CMC medium and incubated at room temperature (27°C) for 9 hours using a shaking incubator at 120 rpm (OD 0.6-0.8). The bacterial culture was measured using a spectrophotometer at 600 nm. The remaining sample was centrifuged at 10,000 rpm for 10 minutes. A supernatant containing a crude enzyme was taken to be tested for cellulase enzyme activity. Protein concentration was measured using the Bradford method, using bovine serum albumin (BSA) as a standard.

The cellulase activity test of the isolate was done by measuring the reduction of sugar by cellulase using the Bernfeld method. Cellulase activity testing was carried out on the crude enzyme of bacterial isolates with three treatments: enzyme sample, control, and blank, using dinitrosalicylic acid (DNS) reagent (Miller, 1959). A total of 1 mL of enzyme was mixed with 1 mL of 1% CMC solution in 0.05 M phosphate buffer. The enzyme sample was then incubated at 27°C for 5, 10, 20, 30, and 40 minutes and added with 2 mL of DNS solution. Control was made with 1 mL of 1% CMC solution in 0.05 M phosphate buffer, which was then added with 2 mL of DNS and 1 mL of the enzyme after incubating at 27°C for 5, 10, 20, 30, and 40 minutes. Blank was made with 1 mL of 1% CMC solution in 0.05 M phosphate buffer with 2 mL of DNS added after incubating at 27°C for 5, 10, 20, 30, and 40 minutes. The enzyme samples, controls, and blanks were then heated in boiling water for 5 minutes and cooled for 10 minutes. Enzyme activity was determined by measuring the absorbance of the three treatments at a wavelength of 550 nm. One unit of enzyme activity is defined as the amount of enzyme that produces 1 µmol of glucose per minute.

Identification of cellulolytic bacteria using morphological and biochemical characterization

Biochemical characterization of bacterial physiological tests was carried out on potential bacterial isolates previously characterized by cells (gram-positive or negative) and endospores (present/absent). Based on these characterizations, bacterial isolates are tested physiologically following Bergey's Manual of Determinative Bacteriology. Physiological tests performed include acid-fast stain, catalase test, and a starch hydrolysis test.

Result and Discussion

Soil bacteria have been known to have great potential in producing functional natural components, one of which is by producing the enzyme cellulase. Isolation is the initial stage of obtaining cellulolytic bacteria. Isolation of cellulolytic bacteria using CMC media aims to select and obtain bacteria that are able to break down cellulose into smaller reducing sugars as a carbon source. Based on the isolation results, 5 bacterial isolates were isolated from soil samples, and their morphological characteristics can be seen in Table 1.

Table 1. Characteristics of bacterial colony morphology

Isolate code	Shape	Size	Surface	Color	Optical Characterization	Elevation	Margin	Texture
ACI1	Irregular	Medium	Dull	Cream	Translucent	Flat	Undulate	Moist
ACI2	Circular	Tiny	Dull	White	Opaque	Raised	Entire	Moist
ACI3	Irregular	Tiny	Dull	White	Opaque	Flat	Entire	Moist
AJS1	Irregular	Medium	Dull	White	Cloudy	Flat	Undulate	Moist
AJS2	Circular	Tiny	Dull	White	Opaque	Raised	Entire	Moist

Table 2. Characteristics of bacterial cell morphology

Isolate code	Gram's reaction	Shape	Arrangement	Endospore
ACI1	Negative	Rod	Single	Absent
ACI2	Negative	Rod	Single	Absent
ACI3	Positive	Rod	Single	Absent
AJS1	Positive	Rod	Strepto	Absent
AJS2	Negative	Rod	Diplo	Absent

Research by Arju Hossain *et al.* (2021) and Sahu *et al.* (2024) respectively showed that the isolation of cellulolytic bacteria from soil using 0.5-1% CMC media successfully obtained isolates that have the potential to degrade cellulose. In addition, research by Nababan *et al.* (2019) revealed that CMC media is a substrate and inducer for producing crude cellulase enzymes by cellulolytic bacteria. Based on Table 1, the colony morphology of isolate AC1 has almost morphological similarities with isolate AJS1 in terms of shape, size, surface, elevation, edges, and texture. While isolates ACI2, ACI3, and AJS2 have similarities in size, surface, color, optical characteristics, edges, and texture. The purified bacterial colonies were characterized for cell morphology using Gram staining and endospore staining, as shown in Table 2.

Gram staining is called differential staining because it can distinguish a group of bacteria from other groups based on cell wall structure and overall cell shape (Moyes *et al.*, 2009). Gram-positive bacteria can hold the primary dye complex, crystal violet, so the cells will appear dark blue or purple. Gram-negative bacteria will lose the crystal violet complex after being rinsed with alcohol and will be colored by the counterstain safranin so that the cells will appear red. Gram-negative bacteria have thin peptidoglycan walls and do not contain toxic acid, so they experience color decolorization (Paray *et al.*, 2023). Gram staining results show that isolates ACI1, ACI2, and AJS2 are gram-negative rod-shaped bacteria (Figure 1A; Figure 1B; Figure 1E). ACI3 isolates are gram-positive, rod-shaped bacteria (Figure 1C), while AJS1 isolates are gram-positive bacteria in the form of chained rods or streptobacill (Figure 1D). Endospore staining was performed on two isolates of Gram-positive bacteria, namely isolates ACI3 and AJS1. Based on the results of endospore staining, the two isolates only showed vegetative cells, and no spores were found (Figure 2).

Endospores are only owned by certain groups of bacteria, especially in the genera *Bacillus* and *Clostridium*. Endospores are formed at the sporogenesis stage as a mechanism for bacteria to survive in unsuitable environments, such as nutrient deficiencies, pH effects, differences in carbon and nitrogen sources, high heating, and chemical concentrations (Jiang *et al.*, 2020). Research shows that the vegetative cells of *Bacillus* isolates cultured for more than 48 hours can form endospores in response to a lack of nutrients. This is due to the thick and hard spore sheath, so sufficient heat is needed so that the malachite green dye can penetrate the endospores. However, neither test isolate showed endospore formation.

The five bacterial isolates were tested qualitatively by forming a clear zone in media treated with Congo red dye. A qualitative test is used to confirm the presence of the cellulase enzyme using a preliminary screening method (Porkavi *et al.*, 2021). Qualitative test of cellulose enzyme uses Congo red dye that can interact with β-1,4 glycosidic bonds in CMC media. Giving NaCl causes Congo red that does not interact to be washed, so that a clear color is produced and calculated as a cellulolytic index (Demissie *et al.*, 2024). Isolates AC1, ACI2, AJS1, and AJS2 showed the ability to produce cellulase, as indicated by the formation of clear zones around bacterial colonies (Figure 3A). Based on the cellulolytic index value, it can be seen that the isolates that produce the largest clear zone are isolates AJS1 and AJS2 (Table 3). Isolates AJS1 and AJS2 produce a very clear zone (Figure 3b), and each clear zone's index is 1.25 and 0.60. Meanwhile, isolates ACI1 and ACI2 have a low level of zone clarity and murdy appearance (Figure 3C).

Allium spp. has shown the ability to inhibit both Gram-positive and Gram-negative bacteria. This highlights its strong potential as an alternative or adjunct to antibiotics, especially amid the rise of resistant bacterial strains. Moreover, the presence of other bioactive compounds in *Allium spp.* such as diallyl disulfide, ajoene, and quercetin enriches the antibacterial profile of these plants, making them ideal candidates in the development of nature-based antimicrobial therapies. With the growing problem of antibiotic resistance worldwide, the potential of *Allium spp.* to inhibit gram- negative and positive bacteria offers a promising solution. Further development of the therapeutic applications of *Allium spp.* may pave the way for the discovery of new, safer and more effective antibiotics, which are urgently needed in today's medical world (Rahmi *et al.*, 2019).

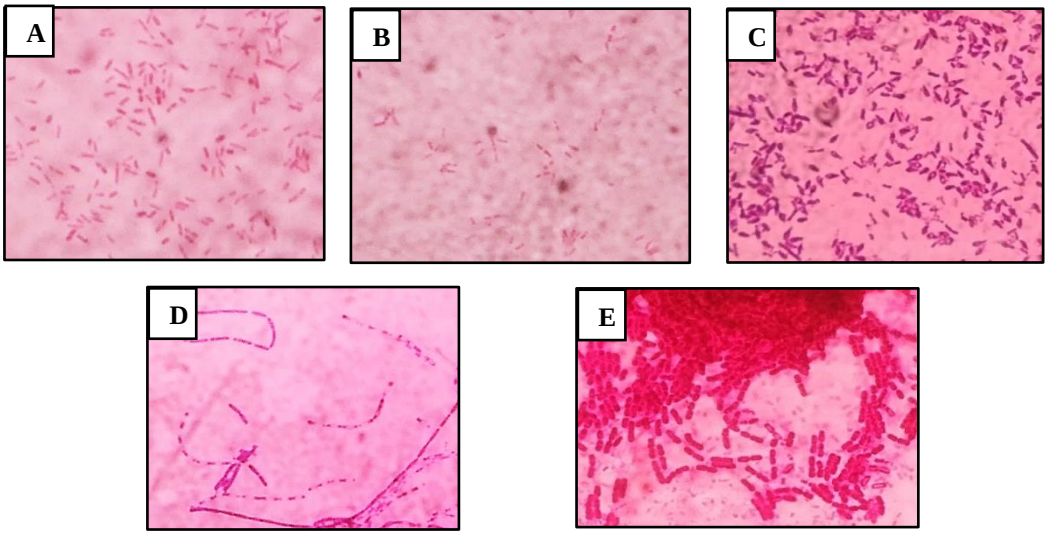

Figure 1. Gram-staining results of isolate: (A) ACI1; (B) ACI2; (C) ACI3; (D) AJS1; and (E) AJS2 with magnification of 1000x

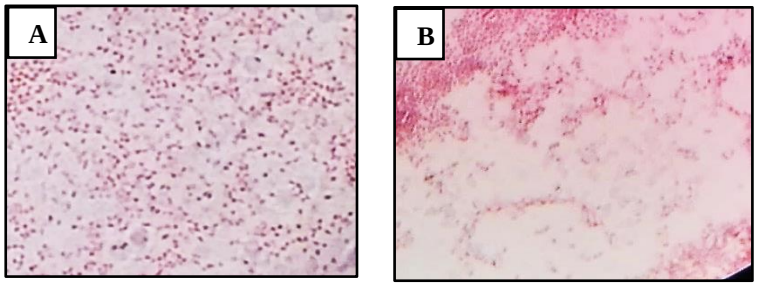

Figure 2. Endospore-staining results of isolate: (A) ACI3; and (B) AJS1with magnification of 1000x

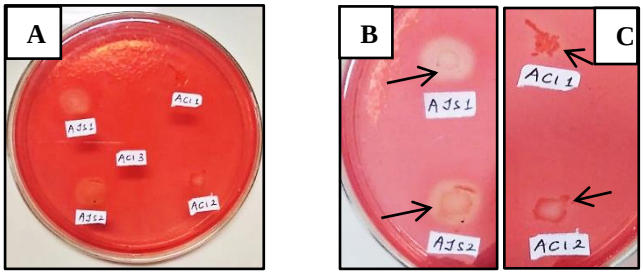

Figure 3. Qualitative cellulolytic activity: (A) the clear zone of five bacterial isolates; (B) the clear zone (black arrow) around the bacterial colony of isolate AJS1 and AJS2; and (C) isolate ACI1 and ACI2

Table 3. The cellulolytic index from five bacterial isolates

Isolate code	The clear zone appearance	The average clear zone diameter (mm)	The average bacterial colony diameter (mm)	Cellulolytic index
ACI1	+	7,00	6,25	0,12
ACI2	+	7,75	6,75	0,15
ACI3	-	-	-	-
AJS1	+++	11,25	5,00	1,25
AJS2	+++	11,25	7,00	0,60

Note: (+) = indicates a clarity level

Table 4. Cellulose enzyme activity of bacterial isolate AJS1

Incubation time (minute)	Total protein amount(mg/ml)	Glucose concentrate (mg/ml)	Unit Activity (U/ml)	Specific activity (U/mg)
5	0,275	0,003	0,0034	0,012
10	0,275	0,009	0,0049	0,018
20	0,275	0,005	0,0015	0,005
30	0,275	0,005	0,0009	0,003
40	0,275	0,004	0,0005	0,002

The cellulolytic index shows the value of extracellular cellulase activity produced by test bacterial isolates hydrolyzing cellulose in CMC media. The higher the cellulolytic index value, the higher the cellulase enzyme activity qualitatively. According to Demissie *et al.* (2024), cellulase enzyme activity can be categorized based on the cellulolytic index (CI) value, namely the low category if CI < 1, the medium category if CI = 1 to 2, and the high category if CI> 2. Based on this category, isolate AJS1 has a medium cellulolytic index value but is higher than other isolates, so it is used for further tests.

The principle of cellulase enzyme activity testing is to measure reducing sugar in the form of glucose. Protein content and glucose concentration were measured to determine enzyme activity. The protein amount of the crude extract of the extracellular enzyme sample was 0.275 mg/mL (Table 4).

In this study, CMC was used as a medium to induce cellulase production by isolating AJS1. The cellulase enzyme hydrolyzes cellulose into simple sugar, namely glucose, so the higher the cellulase enzyme activity, the more glucose is produced. Cellulase activity is mainly produced in the growth phase (logarithmic), so a decrease in cellulase activity will occur if it passes its growth phase. The bacteria were grown for 9 hours until an absorbance value of 0.921 was obtained. These results indicate that the bacterial isolate has passed its logarithmic phase (0.2-0.8). Based on research by Kurniawati *et al.* (2021), the highest cellulase activity was produced by *Serratia marcescens* bacteria in the logarithmic phase to the beginning of the stationary phase, which is in the incubation time range of 8 to 16 hours.

The glucose concentration produced was relatively stable until the 40th minute of incubation and was highest at the 10th minute of incubation (Table 4). The unit activity value and specific cellulase activity increased at the 10th minute of incubation but then decreased until the 40th minute. This result indicates that the enzyme activity is not stable. The highest unit and specific cellulase activity values of AJS1 were 0.0049 U/mL and 0.018 U/mg, respectively. This result is lower than the research conducted by Gatpatan *et al.* (2024), which amounted to 0.41 U/mg. This is due to cellulase activity, which can be influenced by several factors, including pH, the use of CMC concentration as a carbon source, temperature, and the addition of nitrogen sources, inoculum size, and incubation period (Fouda *et al.*, 2024). Research by Islam & Roy (2018) showed that *Paenibacillus* sp. strain incubated in 1.0% CMC medium produced cellulase of 0.9 µmol ml^{-1} min^{-1} at pH 7.0 and temperature of 40°C after 24 hours of incubation.

Table 5. The identification process of isolate AJS1 based on Bergey's Manual

Identifying process parameters	AJS1	*Bacillus* spp.	*Corynebacterium* spp.	*Lactobacillus* spp.	*Mycobacterium* spp.
Cell wall type	Gram Positive	Gram Positive	Gram Positive	Gram Positive	Gram Positive
Cell shape	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Endospore	-	+	-	-	-
Acid-fast stain	-	x	-	-	+
Catalase	+	x	+	-	x
Starch hydrolysis	+	x	+	x	x

Note: Not conducted (x).

The potential bacterial isolate, AJS1, is known to be a gram-positive, rod-shaped bacterium without endospores. The determination of biochemical characters based on these three characteristics follows Bergey's Manual of Determinative Bacteriology. Isolate AJS1 was tested physiologically using the acid-fast stain, catalase, and starch hydrolysis tests (**Table 5**). Isolate AJS1 showed positive results against acid staining. AJS1 isolates tested for catalase-formed air bubbles after reacting with H_2O_2. After the starch hydrolysis test, this isolate produced a clear zone. The results of the starch hydrolysis test are the last test of gram-positive rods without endospores, so it can be assumed that isolate AJS1 is *Corynebacterium* spp.

Corynebacterium spp. is aerobic, so it is in accordance with the positive results shown from the catalase test. These bacteria are non-pathogenic and categorized as industrial microorganisms due to their use in the food and feed industry (Lin *et al.*, 2022). One of these bacterial species, *Corynebacterium glutamum*, can be isolated from soil and used as a bioremediation agent (Ray *et al.*, 2022). In addition, *Corynebacterium glutamum* bacteria are also known to produce cellulase enzymes that can degrade lignocellulosic materials as a source of energy (Kim *et al.*, 2014). This follows the research on isolate AJS1, which has been proven qualitatively and quantitatively to produce cellulase enzymes. However, molecular identification is necessary to determine the bacteria more precisely.

Conclusions

The isolated bacteria from soil consisted of 5 isolates, namely ACI1, ACI2, ACI3, AJS1, and AJS2. Isolates ACI1, ACI2, and AJS2 are gram-negative rod bacteria, isolate ACI3 is a gram-positive rod bacteria, while isolate AJS1 is a gram-positive rod bacteria in chains. No endospores were produced from the gram-positive isolates tested. Qualitative test results in the form of clear zones and cellulolytic index of the five isolates showed that isolate AJS1 has the most potential with a cellulolytic index of 1.25. Quantitative test results of unit activity and specific activity of AJS1 cellulase enzyme were highest at 0.0049 U/ml and 0.018 U/mg produced for 10 minutes, respectively. Based on biochemical tests following Bergey's Manual of Determinative Bacteriology, it can be assumed that isolate AJS1 is a *Corynebacterium* spp.

References

Abdella, M. A. A., Ahmed, N. E., & Hasanin, M. S. (2024). Green ecofriendly enhancement of cellulase productivity using agricultural wastes by Aspergillus terreus MN901491: statistical designs and detergent ability on cotton fabrics. *Microbial Cell Factories* 23(1): 1–13.

Akhtar, N., & Goyal, D. (2015). Biosurfactants and Bioemulsifiers for Treatment of Industrial Wastes. *Advances in Biodegradation and Bioremediation of Industrial Waste* 2015: 141–168.

Arju Hossain, Md., Akash Ahammed, Md., Islam Sobuj, S., Kubra Shifat, S., & Dipta Somadder, P. (2021). Cellulase Producing Bacteria Isolation, Screening and Media Optimization from Local Soil Sample. *American Journal of Microbiological Research* 9(3): 62–74.

Bamrungpanichtavorn, T., Ungwiwatkul, S., Boontanom, P., & Chantarasiri, A. (2023). Diversity and cellulolytic activity of cellulase-producing bacteria isolated from

the soils of two mangrove forests in Eastern Thailand. *Biodiversitas* 24(7): 3891–3902.

Bautista-Cruz, A., Aquino-Bolaños, T., Hernández-Canseco, J., & Quiñones-Aguilar, E. E. (2024). Cellulolytic Aerobic Bacteria Isolated from Agricultural and Forest Soils: An Overview. *Biology* 13(2): 1-22.

Benalaya, I., Alves, G., Lopes, J., & Silva, L. R. (2024). A Review of Natural Polysaccharides: Sources, Characteristics, Properties, Food, and Pharmaceutical Applications. *International Journal of Molecular Sciences* 25(2): 1-32.

Borthakur, I., Devi, R. P., Karthikeyan, S., Ramesh, D., & Murugananthi, D. (2024). Microbial Cellulase Production: Current Technologies and Future Prospects. *Journal of Pure and Applied Microbiology* 18(4): 2188–2204.

Demissie, M. S., Legesse, N. H., & Tesema, A. A. (2024). Isolation and characterization of cellulase producing bacteria from forest, cow dung, Dashen brewery and agro-industrial waste. *PLoS ONE* 19(4 April): 1–12.

Ejaz, U., Sohail, M., & Ghanemi, A. (2021). Cellulases: From bioactivity to a variety of industrial applications. *Biomimetics* 6(3): 1–11.

Fouda, A., Alshallash, K. S., Atta, H. M., El Gamal, M. S., Bakry, M. M., Alawam, A. S., & Salem, S. S. (2024). Synthesis, Optimization, and Characterization of Cellulase Enzyme Obtained from Thermotolerant Bacillus subtilis F3: An Insight into Cotton Fabric Polishing Activity. *Journal of Microbiology and Biotechnology* 34(1): 207–223.

Fu, Z. H., Liu, J., Zhong, L. Bin, Huang, H., Zhu, P., Wang, C. X., & Bai, X. P. (2022). Screening of cellulose-degrading yeast and evaluation of its potential for degradation of coconut oil cake. *Frontiers in Microbiology* 13(October): 1–14.

Fu, Z., Zhong, L., Tian, Y., Bai, X., & Liu, J. (2024). Identification of Cellulose-Degrading Bacteria and Assessment of Their Potential Value for the Production of Bioethanol from Coconut Oil Cake Waste. *Microorganisms* 12(2): 1-15.

Gatpatan, I. G. T., Cabulong, R. B., & Sadaba, R. B. (2024). Diversity and Screening of Cellulolytic Microorganisms from Mangrove Forests, Natural Parks, Paddy Field, and Sugarcane Plantation in Panay Island, Philippines. *International Journal of Microbiology 2024*: 1-16.

Islam, F., & Roy, N. (2018). Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. *BMC Research Notes* 11(1): 1–6.

Jiang, L., Jia, G., Wang, Y., & Li, Z. (2020). Optimization of Sporulation and Germination Conditions of Functional Bacteria for Concrete Crack-Healing and Evaluation of their Repair Capacity. *ACS Applied Materials and Interfaces* 12(9): 10938–10948.

Kamali, E., Jamali, A., Izanloo, A., & Ardebili, A. (2021). In vitro activities of cellulase and ceftazidime, alone and in combination against Pseudomonas aeruginosa biofilms. *BMC Microbiology* 21(1): 1–10.

Kim, S. J., Hyeon, J. E., Jeon, S. D., Choi, G. wook, & Han, S. O. (2014). Bi-functional cellulases complexes displayed on the cell surface of corynebacterium glutamicum increase hydrolysis of lignocelluloses at elevated temperature. *Enzyme and Microbial Technology* 66: 67–73.

Kurniawati, L., Kusdiyantini, E., & Wijanarka, W. (2021). Pengaruh Variasi Suhu Dan Waktu Inkubasi Terhadap Aktivitas Enzim Selulase Dari Bakteri Serratia marcescens. *Bioma : Berkala Ilmiah Biologi* 23(1): 33–42.

Lin, K., Han, S., & Zheng, S. (2022). Application of Corynebacterium glutamicum engineering display system in three generations of biorefinery. *Microbial Cell Factories*, 21(1): 1–12.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry* 31(3): 426–428.

Monika, K., & Rukhaya, S. (2025). Enzymes at Work: Eco-Friendly Solutions for Modern Textile Finishing. *Vigyan Varta An International E-Magazine for Science Enthusiats* 6(1): 178–186.

Moyes, R. B., Reynolds, J., & Breakwell, D. P. (2009). Differential staining of bacteria: Gram stain. *Current Protocols in Microbiology, SUPPL.* 15: 1–8.

Nababan, M., Gunam, I. B. W., & Mahaputra Wijaya, I. M. (2019). Produksi Enzim Selulase Kasar Dari Bakteri Selulolitik.

Jurnal Rekayasa Dan Manajemen Agroindustri 7(2): 190.

Paray, A. A., Singh, M., & Amin Mir, M. (2023). Gram Staining: A Brief Review. *International Journal of Research and Review* 10(9): 336–341.

Porkavi, B., Kalaiselvi, P., Davamani, V., Anandham, R., & Maheswari, M. (2021). Qualitative and quantitative assessment of cellulolytic enzyme production in the microbial isolates from environmental samples. *The Pharma Innovation* 10(2): 127–131.

Putri, A. L., & Setiawan, R. (2019). Isolation and screening of actinomycetes producing cellulase and xylanase from Mamasa soil, West Sulawesi. *IOP Conference Series: Earth and Environmental Science* 308(1): 5–11.

Rahman SU, Ra. M. S. H. Z. N. Z. S. A. A. B. I. F. (2021). Microbial Cellulases: A Review on Strain Development, Purification, Characterization and their Industrial Applications. *Journal of Bacteriology and Mycology* 8(5): 1-5.

Ranganathan, S., Mahesh, S., Suresh, S., Nagarajan, A., Z. Sen, T., & M.Yennamalli, R. (2022). Experimental and computational studies of cellulases as bioethanol enzymes. *Bioengineered*, 13(5): 14028–14046.

Ray, D., Anand, U., Jha, N. K., Korzeniewska, E., Bontempi, E., Proćków, J., & Dey, A. (2022). The soil bacterium, Corynebacterium glutamicum, from biosynthesis of value-added products to bioremediation: A master of many trades. *Environmental Research* 213(May): 1-12.

Sahu, T., Choudhary, R., & Kulkarni, P. (2024). Isolation and Screening of Cellulase Producing Bacteria from Sugar Industry Waste. *Research Journal of Agricultural Sciences* (July): 918-921.

Teather, R. M., & Wood, P. J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and Environmental Microbiology* 43(4): 777–780.

Wang, J., Bao, F., Wei, H., & Zhang, Y. (2024). Screening of cellulose-degrading bacteria and optimization of cellulase production from Bacillus cereus A49 through response surface methodology. *Scientific Reports*, 14(1): 1–14.

Yunus, G., & Kuddus, M. (2021). Cold-Active Microbial Cellulase : Novel Approach To Understand Mechanism And Its Applications In Food And Beverages Industry. *Journal of Microbiology, Biotechnology and Food Sciences* 10(4): 524–530.