Vol. 13(3): 796-811, September 2025 DOI: https://doi.org/10.23960/jsl.v13i3.1238

Jurnal Sylva Lestari

P-ISSN: 2339-0913
E-ISSN: 2549-5747

Journal homepage: https://sylvalestari.fp.unila.ac.id

Full Length Research Article

Isolation, Characterization, and Identification of Cellulolytic Bacteria Colony from Forest and Landfill Environments in Iligan City, Philippines

Jessa Marie Buniel Sabado*

Department of Environmental Science, College of Forestry and Environmental Science, Caraga State University, Ampayon, Butuan City, Philippines
* Corresponding author. E-mail address: jbsabado@carsu.edu.ph

ARTICLE HISTORY:

Received: 30 June 2025
Peer review completed: 23 July 2025
Received in revised form: 15 August 2025
Accepted: 29 August 2025

KEYWORDS:

Biomass utilization
Cellulolytic bacteria
Cellulose-degrading
Forest environment
Landfill environment

ABSTRACT

The global proliferation of landfills represents a significant environmental challenge, as the generation and accumulation of solid waste continue to increase at an accelerating rate each year. This study aimed to isolate and characterize cellulose-degrading bacteria from forests in Barangay Suarez and landfills in Barangay Bonbonon and Barangay Santiago, Iligan City. Soil samples were randomly collected, and bacterial isolation was performed through serial dilution and spread plating on nutrient agar supplemented with nystatin as an antifungal agent. Pure cultures of the isolated colonies were obtained by streak plating and screened for cellulose-degrading activity using cellulose agar. These isolates were then subjected to morphological and biochemical tests for characterization and identification. Results showed that 18.42% of bacterial isolates tested positive for cellulolytic activity. Based on the observed characteristics, five isolates were identified as *Bacillus*, four as *Pseudomonas*, two as *Cellulomonas*, and three as *Arthrobacter*. Cellulolytic degrading bacteria provide valuable information for future technological applications, particularly in utilizing biomass waste. A 16S rRNA test for the bacterial isolates is highly recommended, as it provides more detailed and precise characterization and identification of the strain at the species level.

1. Introduction

Forest soils provide physical support, moisture, and nutrients for plant and animal life. Forest soil also acts as a natural filter, breaking down natural waste, including toxic substances, making forests essential to sustaining life. This offers both direct and indirect benefits to the environment and all living organisms (Rahmadwiati et al. 2022). Many minerals found in forest soils are crucial in maintaining the structural and functional integrity of both fauna and flora, which significantly contributes to supporting the forest cover (Binkley and Fisher 2019). However, any changes in soil denaturing increase the microbial stress. This trend has substantial implications for the abundance and functional diversity of microbial communities (Bogati and Walczak 2022). It could lead to slight influences on the microbial community structure (Chang et al. 2017).

Landfills pose a significant global environmental concern, as the volume and impact of solid waste are increasing rapidly worldwide (Mor and Ravindra 2023). Approximately 1.3 billion tons of solid waste are collected in cities worldwide each year (Valavanidis 2023). The widespread

presence of unmanaged dumpsites has significantly contributed to the degradation of natural resources. Additionally, the lack of proper waste segregation results in the mixing of various waste types in landfills, posing serious threats to both human health and ecological integrity.

Microorganisms play a crucial role in the biogeochemical cycling of elements, which can be beneficial for environmental and industrial purposes (Kaksonen 2018). Especially, decomposers are microorganisms that release enzymes to break down compounds and create useful biomass. In the process of decay from dead animals and plants, these microorganisms decompose (Khatoon et al. 2017). One of the decomposers is cellulolytic bacteria, which are cellulose-degrading bacteria abundant in soil, even in small amounts, where they contain millions of decaying organic matter (Bhatia et al. 2024). They utilized carbon as their primary energy source, nitrogen to synthesize proteins, and excreted nutrients from plants to sustain their survival (University of Illinois Extension 2018). Cellulose, a primary structural component of the plant cell wall, is one of the most abundant organic compounds on earth. It is widely distributed in plants and trees, with an estimated global production of approximately 180 billion tons per year (Butnariu and Flavius 2022). It is composed of linear homopolymer β (1-4) linked hydrolyzed polysaccharide, a polymer of glucose that is linked by a glycosidic bond (Nasrollahzadeh et al. 2021). Cellulose has a wide range of applications in both agricultural and industrial sectors. It is used in the production of paper, textiles, cotton, and various fibers to meet human needs. Industrially, cellulose serves as a renewable source of energy, playing a key role in biofuel production through processes such as sugar fermentation to produce ethanol (Sundarraj and Ranganathan 2018).

Cellulolytic microorganisms are commonly observed on the forest floor (Bautista-Cruz 2024). The biomass accumulated due to leaf shedding from a heterogeneous tree population permits the rapid transformation of the raw material through the action of different groups of microorganisms (Kozlowski et al. 2012). The primary cellulose-utilizing species include aerobic and anaerobic hemophilic bacteria, filamentous fungi, basidiomycetes, thermophilic bacteria, and actinomycetes. The amount, type and availability of organic matter determine the size and population of microorganisms in the soil system (Bautista-Cruz 2024). There is an increasing industrial focus on the development of innovative and cost-effective technologies for the conversion of biomass into sustainable bioenergy (Yamakawa et al. 2018). According to Harindintwali et al. (2022), cellulolytic bacteria are a promising feedstock for energy production and the production of value-added chemicals, offering a renewable solution to modern waste disposal issues and reducing dependence on fossil fuels by generating energy in the form of glucose.

However, improper waste segregation reduces microbial activity, leading to slower decomposition rates. Human waste is generally categorized into biodegradable and non-biodegradable types, where recycling plays a crucial role in their separation and management. Effective recycling minimizes the volume of waste directed to landfills and enhances the efficiency of microbial decomposition. Proper segregation not only supports microbial processes but also contributes to public health, industrial efficiency, and environmental cleanliness. This study aims to isolate, characterize and identify cellulolytic bacteria colonies from forest and landfill environments. However, this study is limited only to landfills and forest environments.

2. Materials and Methods

2.1. Location of the Study

These sampling sites were chosen to isolate waste-degrading microorganisms, such as bacteria, where biodegradable waste was found and consumed as their food source for growth and propagation (Masngut et al. 2017). Iligan City is located in the northern part of Mindanao, with a total population of 322,821 as of 2010. The land area of the city is 813.37 km² (314.04 sq mi), comprising 44 barangays. There are three sampling sites: Barangay Bonbonon and Barangay Santiago for the landfill area, and Barangay Suarez for the forest area. At each site, three points were randomly chosen for soil sampling.

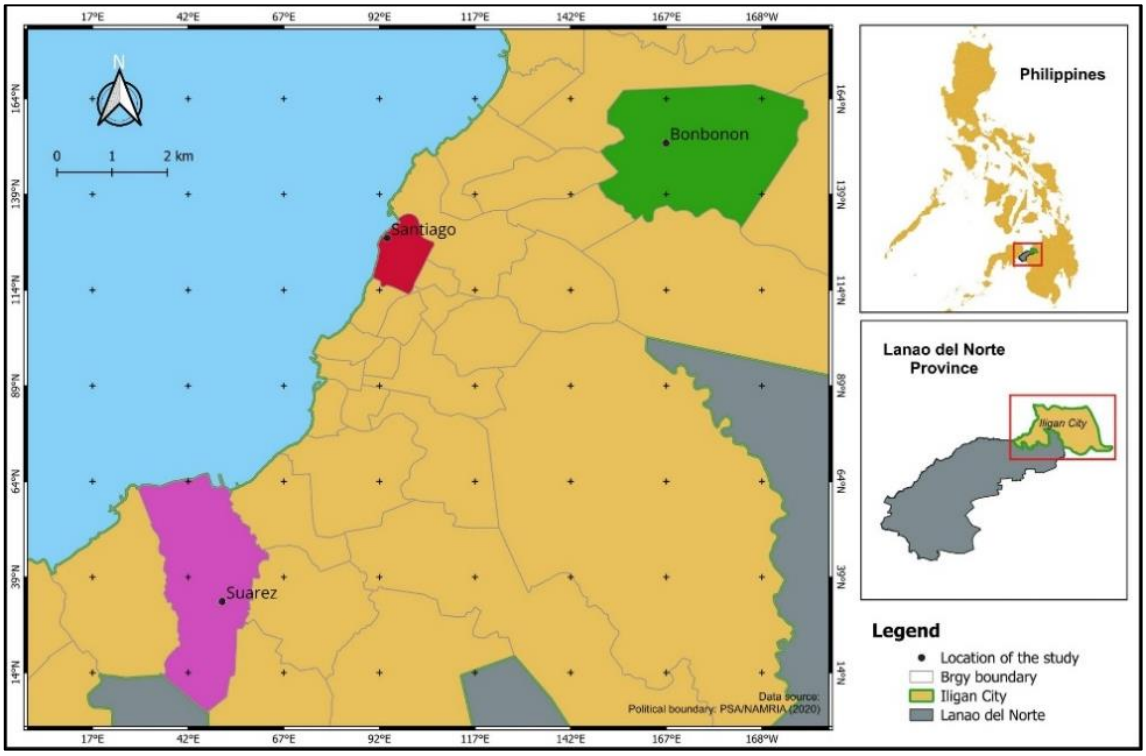


Fig. 1. Location of three sampling sites in Iligan City, Philippines.

2.2. Soil Sample Collection

Soil samples were randomly collected from three different sampling points at each of the following sites: Barangay Bonbonon, Santiago, and Suarez in Iligan City. Samples were taken from the surface layer of the soil, and any biodegradable or non-biodegradable materials mixed with the soil were carefully removed. The cleaned soil samples were then placed in autoclavable plastic containers and transported to the laboratory for isolation, morphological characterization and biochemical characterization.

The samples were measured using the digital scale. Approximately 10 grams of soil were measured for each of the three sampling points (Arshad et al. 1997). The weighted samples were placed into a 100 mL sterile beaker, and 90 mL of distilled water was added. The solutions were then thoroughly mixed until a suspension was observed. The prepared supernatant was used for serial dilution (Thomas 2015).

2.3. Isolation

2.3.1. Serial dilution method

Sterile test tubes were prepared containing 9 mL of distilled water and labeled as A, B, C, and D. 1 mL of diluted soil was aseptically transferred into test tube A. The tube was then gently mixed to ensure good distribution and break up clumps of bacteria. Another 1 mL was aseptically transferred from tube A to tube B and mixed. The same process was applied for tubes C and D. Test tube D was used for the spread plate method. The serial dilution method is a microbiological technique used to reduce a dense population of microorganisms to a countable concentration. This method involves the stepwise dilution of a bacterial suspension, typically in a 1:10 ratio, by transferring a fixed volume of the sample into a series of sterile diluents (Cullen and MacIntyre 2016).

2.3.2. Spread plate method

This technique utilizes prepared liquid culture media to isolate bacteria present in soil samples and is commonly employed to study soil microbial communities (Cormier and Janes 2014). After the dilution, 0.1 mL from tube D was transferred into a fresh nutrient agar plate and spread aseptically using a sterile L-rod to equalize the distribution of bacteria. The nutrient agar composition includes 0.5% peptone, which provides organic nitrogen. 0.3% beef extract/yeast extract – the water-soluble content of these extracts contributes vitamins, carbohydrates, nitrogen, and salts 1.5% agar (Devika et al. 2021). The plates were then incubated at room temperature for 48 hours. The isolated colonies were used in the streak plate method.

2.3.3. Streak plate method

The streak plate technique is a method used to obtain well-isolated colonies from a mixed bacterial culture, allowing for the development of pure culture isolation. It is commonly used in microbiology to separate individual bacterial isolates. Using a sterile inoculating loop, the sample is streaked across the surface of an agar plate in a specific pattern, gradually thinning out the bacterial isolates so that individual colonies can form (Patra et al. 2020). Nutrient agar plates were prepared and divided into four quadrants. A sterile inoculating loop was used to streak the isolates. The plate was incubated at room temperature for 24 hours. The isolated colonies in the streak plate method were transferred aseptically to an agar slant and incubated for 24 hours at room temperature. The pure culture was maintained and used for determining cellulose activity.

2.3.4. Nystatin

A 0.1 mL drop of nystatin was added to every media used for biochemical tests to inhibit the growth of fungi that can affect the bacterial colony (Nosratzehi et al. 2019). The discovery of nystatin in 1950 marked the beginning of antifungal therapy, soon followed by the introduction of amphotericin B, another breakthrough in the treatment of fungal infections. The development of antifungal agents represents a critical milestone in the global effort to combat medical mycoses. However, despite these advancements, fungal infections remain an underrecognized and increasingly prevalent public health threat, positioning them as a significant and emerging global health concern (Sousa et al. 2023).

2.4. Determination of Cellulolytic Activity

2.4.1. Cellulose agar

Cellulose agar media composed of 0.5 g KH_2PO_4, 0.25 g $MgSO_4$, 2.0 g cellulose, 15 g agar, 2 g gelatin, and 1 L distilled water at pH 6.8–7.2 (Nonaka et al. 2014). The pure culture was inoculated and incubated in a laminar airflow for 48 hours at room temperature. The presence of a clear zone determines the cellulose-degrading activity of the isolated bacteria. A clear zone is a distinct area surrounding microbial colonies on an agar plate, indicating the degradation of the substrate or the activity of the microorganisms. It is commonly observed during assays for enzymatic activity, antimicrobial susceptibility testing, or studies of microbial interactions.

2.5. Morphological Characterization of the Isolation

2.5.1. Gram staining

A drop of distilled water was placed in a clean glass slide. Using the inoculating loop, a colony is picked from the plated pure culture and transferred to the slide, then mixed, and left to air dry. The smear was then heat-fixed by passing the glass slide through the flame three times. The smear was flooded with crystal violet, the primary stain, and allowed to stand for 1 minute; then, it was rinsed with distilled water. A few drops of gram's iodine were added to the smear and allowed to stand for another minute, then it was washed with distilled water. Then, a decolorizer was placed on the slide for 10 seconds and immediately washed with distilled water. The smear was counterstained with safranin for 1 minute, then washed with distilled water and air-dried. The slide was viewed using the oil immersion microscope. To increase the resolving power of the microscope, immersion oil was used. The gram reaction of the isolate, shape, and arrangement were noted (Paray 2023).

2.6. Biochemical Characterization of the Isolation

2.6.1. Determination of oxygen requirement

Determining the oxygen requirement is one way to identify a microorganism. Microorganisms can be classified as either aerobic, which require oxygen for growth, or anaerobic, which thrive in an environment without oxygen. In determining the oxygen requirement of an organism, fluid thioglycollate medium is commonly used (Sakhno et al. 2018). A 10 mL of thioglycollate broth was prepared and inoculated with the unknown bacteria using an inoculated loop by swirling into the bottom of the medium. The prepared culture was observed after 48 hours at room temperature. The growth on the upper surface of the medium indicates that the organism is an aerobe, while growth at the bottom is indicative of an anaerobic organism, and growth throughout the medium is indicative of a facultative anaerobe.

2.6.2. Catalase test

A colony from the prepared culture was transferred to a clean glass slide, and 0.1mL drops of 3% hydrogen peroxide were added to the slide. The formation of bubbles indicates a positive test for breaking down hydrogen peroxide (Virtual Amrita Laboratories Universalizing Education 2018).

2.6.3. Citric acid utilization

Simmon's citrate agar was used, and the slants were in the sterile test tubes. Unknown isolates were transferred by streaking the surface of the medium. The culture was incubated for 48 hours at room temperature. Changes in the color of the medium were observed (Olee 2016).

2.6.4. MacConkey agar

The unknown isolate was streaked into the MacConkey agar using a flame-sterilized loop (Allen 2005). A change in the medium was observed within 48 hours at room temperature. Dark color in the colony is indicative that the bacterium is a lactose fermenter.

2.6.5. Eosin methylene blue (EMB)

An Eosin Methylene Blue (EMB) agar plate was prepared, and the unknown isolates were inoculated onto the plate using a sterile inoculating loop (Ashraf et al. 2024). The plates were then incubated at room temperature for 48 hours. Changes in the color of the isolated colonies were observed.

2.6.6. Mannitol fermentation

A mannitol salt agar (MSA) plate was prepared and divided into four quadrants (De Visscher et al. 2013). The unknown isolates were inoculated using a sterile inoculating loop and then incubated at room temperature for 48 hours. Changes in the color of the medium were observed, as acid production resulting from mannitol fermentation caused the agar's normal color to change to yellow. Mannitol fermenters produced yellow colonies while non-mannitol fermenters produced reddish/purple colonies.

2.6.7. Sulfide-indole-motility

A sulfide-indole-motility (SIM) medium tube was prepared, and the unknown bacterial isolate was inoculated by stabbing the semi-solid medium with a sterile inoculating needle (Idris 2021). The culture was incubated at room temperature for 48 hours. Following incubation, motility was assessed by observing the line of inoculation: a positive motility test was indicated by a diffused growth radiating from the stab line, whereas growth restricted to the stab line indicated a negative result. To assess indole production, 10 drops of Kovac's reagent were added to the culture. A pink to red layer at the top of the medium denoted a positive indole test, while a yellow layer indicated a negative result. For the hydrogen sulfide (H_2S) test, a black precipitate indicated a positive result, whereas no color change signified a negative test.

2.6.8. Methyl red and Voges-Proskauer test

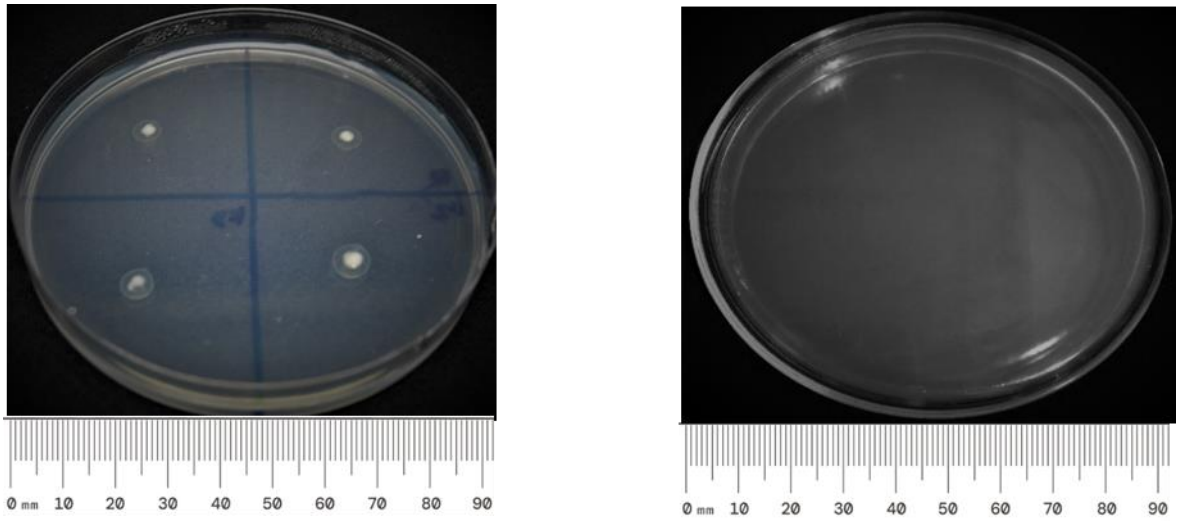
The unknown isolate was inoculated into the prepared MRVP tube and incubated for 48 hours at room temperature (Kones 2020). After 48 hours, 1 mL of the culture was transferred into a sterile tube, and 2 drops of methyl red were added. A red color change is positive, while a yellow color change is negative. To the remaining medium, 10 drops of 5% alpha-naphthol were added and then mixed. This was then followed by the addition of 15 drops of 40% KOH, which was

mixed and allowed to stand for 20 minutes. A change from pink to red is positive, while no change in color is negative.

3. Results and Discussion

3.1. Isolation of Cellulose-Degrading Bacteria

Out of 76 isolated strains from different sampling sites of soil samples, only 14 (18.42%) isolates were found to have the ability to degrade cellulose. For the forest environment, three cellulose-degrading bacteria have been found in Barangay Suarez. For landfill environments, four cellulose-degrading bacteria were found in Barangay Santiago and seven in Barangay Bonbonon. These isolates were tested for their cellulose activity in a medium where cellulose agar is the only carbon source. The formation of a clear zone determined their cellulose-degrading activity, as shown in **Fig. 1**.



(a) (b)

Fig. 1. The formation of a clear zone in cellulose agar isolate 1F2B (a) and control (b) after 2 days of incubation at room temperature.

After two days of incubation, isolates (1F2B, 2F3A, 2F3B, 1S3A, 2S1C, 3S2A, 3S2B, 1B1B, 1B1H, 2B2B, 3B1B, 3B1E, 3B2A, 3B2C) were found to have the ability to degrade cellulose with three replications. In the present study, a smaller number of cellulose-degrading bacteria were observed in the forest environment of Barangay Suarez. Cellulose-degrading bacteria are most commonly seen in the landfill environment of Barangay Bonbonon, followed by Barangay Santiago. The results indicate that landfill environments are favorable sites for isolating cellulolytic bacteria. Cellulolysis is a biological process mediated by the coordinated activity of enzymes within the cellulase system. The enzymatic system comprises three principal classes of soluble extracellular enzymes: endo-1,4-β-glucanase, exo-1,4-β-glucanase (also known as cellobiohydrolase), and β-glucosidase (β-D-glucoside glucohydrolase or cellobiase) (Pradeep and Edison 2022).

Furthermore, cellulolytic bacteria isolated from various environments that can degrade cellulose materials play an essential role in waste degradation (Padhan 2023). The utilization of these materials can help reduce waste generation in the local area (Mostaghimi and Behnamian

2023). Additionally, cellulose-degrading bacteria will be useful in future technological applications, as cellulose is the most abundant biomass on earth. The production of ethanol and biofuels from biomass by fermentation has the greatest potential to resolve both the energetic and environmental demands of bioenergy (Huang et al. 2012).

3.2. Isolate Morphological Characterization

Table 1 presents the cellular characteristics of the bacterial isolates, as determined by gram staining, along with their oxygen requirements, as assessed using thioglycollate broth. The results indicate that ten isolates were gram-positive and exhibited a rod-shaped morphology. These cellular characteristics serve as the primary basis for bacterial identification, as bacteria are generally classified into two major groups: gram-positive and gram-negative. Additionally, four isolates were identified as gram-negative based on their staining reaction. Gram-positive bacteria retain the primary stain crystal violet due to their thick peptidoglycan layer, while gram-negative bacteria retain the counterstain safranin because they have a thin peptidoglycan layer (Paray 2023).

Table 1. Isolates cellular characteristics and their oxygen requirement using thioglycolate broth

Isolate	Gram Stain	Shape	Oxygen Requirement
1F2B	+	*coccobacillus*	obligate aerobe
2F3A	-	*bacillus*	obligate aerobe
2F3B	-	*bacillus*	obligate aerobe
1S3A	+	*staphylococcus*	facultative anaerobe
2S1C	+	*diplobacillus*	obligate anaerobe
3S2A	+	*coccobacillus*	obligate aerobe
3S2B	+	*coccobacillus*	obligate aerobe
1B1B	+	*streptococcus*	facultative anaerobe
1B1H	-	*bacillus*	obligate aerobe
2B2B	+	*diplobacillus*	obligate anaerobe
3B1B	+	*diplobacillus*	obligate anaerobe
3B1E	+	*diplobacillus*	obligate aerobe
3B2A	-	*coccobacillus*	facultative anaerobe
3B2C	+	*bacillus*	obligate anaerobe

Among the fourteen isolates, ten were identified as gram-positive, while four were gram-negative, reflecting the diversity of bacterial cell wall structures present in the soil samples (**Table 1**). The isolates also show the varied cellular morphologies, including *Coccobacillus*, *Bacillus*, *Diplobacillus*, *Staphylococcus*, and *Streptococcus* forms. *Coccobacillus* shapes were observed in isolates 1F2B, 3S2A, 3S2B, and 3B2A, while *Bacillus* forms were typical in 2F3A, 2F3B, 1B1H, and 3B2C. Some isolates, such as 2S1C, 2B2B, 3B1B, and 3B1E, showed *Diplobacillus* shapes, whereas 1S3A and 1B1B were characterized as *Staphylococcus*. In terms of oxygen requirements, the majority of isolates were obligate aerobes, requiring oxygen for growth. At the same time, a few, such as 1S3A, 1B1B and 3B2A, were facultative anaerobes capable of surviving in both aerobic and anaerobic conditions. The test for oxygen requirement is a primary diagnostic tool that differentiates between aerobic, facultative anaerobic, and non-aerobic organisms.

This range of morphological tests suggests a diverse microbial population in the soil, adapted to varying environmental conditions and potentially contributing to different ecological functions such as cellulose degradation. However, morphological identification poses challenges in accurately identifying microbes at the species level (Istikorini and Sari 2022). The growth pattern in a medium can also be used in the diagnostic examination of an unknown organism. **Table 2** shows the morphological characteristics of the isolates based on their growth in nutrient agar, including colony size, shape (configuration), margin, elevation, color, and their presumptive identification based on these features.

Table 2. Morphological characteristics of cellulose-degrading isolates using nutrient agar

Isolate	Size	Configuration	Margin	Elevation	Color	Possible Species
1F2B	Moderate	Round	Entire	Convex	Yellow	*Arthrobacter*
2F3A	Punctiform	Round	Entire	Convex	White	*Pseudomonas*
2F3B	Small	Round	Entire	Mucoid	Yellow	*Pseudomonas*
1S3A	Small	Round	Entire	Mucoid	White	*Cellulomonas*
2S1C	Large	Irregular	Undulate	Flat	Yellow	*Bacillus*
3S2A	Small	Round	Entire	Mucoid	White	*Arthrobacter*
3S2B	Moderate	Irregular	Undulate	Flat	Yellow	*Arthrobacter*
1B1B	Small	Round	Entire	Convex	Yellow	*Cellulomonas*
1B1H	Moderate	Irregular	Undulate	Flat	Yellow	*Pseudomonas*
2B2B	Large	Irregular	Undulate	Flat	Yellow	*Bacillus*
3B1B	Moderate	Irregular	Undulate	Flat	Yellow	*Bacillus*
3B1E	Moderate	Round	Entire	Convex	Yellow	*Bacillus*
3B2A	Small	Round	Entire	Mucoid	Yellow	*Pseudomonas*
3B2C	Moderate	Irregular	Undulate	Flat	Yellow	*Bacillus*

The isolates showed a range of colony morphologies, with sizes varying from punctiform to large, and configurations mostly being round or irregular. Margins were either entire (smooth) or undulate (wavy), and colony elevations ranged from flat to convex and mucoid, indicating differences in surface texture and bacterial secretion. Most colonies were yellow, a typical pigmentation characteristic of many soil bacteria, such as *Bacillus* and *Pseudomonas*, while some colonies were white, commonly associated with *Cellulomonas* and *Arthrobacter*. Based on these observations, isolates were tentatively classified into four genera: *Pseudomonas*, *Bacillus*, *Arthrobacter*, and *Cellulomonas*.

Colony morphology of the isolates in the nutrient agar reveals that the stains 1F2B, 1B1B, 3B1E, are round in form, entire in margin, convex in elevation and color yellow, while stains 2F3B and 3S2A are round entire, mucoid and yellow, then stain 2F3A is round entire convex and color white, stains 1S3A and 3B2A are round, entire, mucoid, white, and lastly stains 2S1C, 3S2B, 1B1H, 2B2B, 3B1B and 3B2C are irregular, undulate, flat, yellow. However, the morphological characteristics of a bacterium were not a solid basis for identifying the unknown. This initial morphological assessment serves as a crucial step in the identification process, providing foundational data before performing biochemical or molecular analyses for more accurate classification. Identification is based more on the reaction of bacteria to a series of biochemical tests, which reveal their physiological requirements.

3.3. Biochemical Characteristics of Cellulose-Degrading Isolates

The physiological requirements of the isolated cellulose-degrading bacteria were determined based on biochemical tests. **Table 3** shows the series of biochemical tests performed. Eosin methylene blue agar and MacConkey agar are both differential and selective media used in the study. Both of the medium inhibits the growth of gram-positive bacteria. Eosin methylene blue agar and MacConkey agar were used to confirm the gram staining results. Presumptive identification of the cellulose-degrading isolates was based on the results of biochemical tests, with morphological characteristics also being observed.

Table 3. Physiological characteristics of cellulose-degrading isolates based on biochemical tests

Isolate	Catalase	SCA	MacConkey	EMB	MSA	SIM			Methyl Red	Voges-Proskauer	Possible Species
						Indole	Motility	Sulfide			
1F2B	+	+	+	+	-	-	+	-	-	-	*Arthrobacter*
2F3A	+	+	-	+	-	-	+	-	+	-	*Pseudomonas*
2F3B	+	+	+	+	-	-	-	-	-	-	*Pseudomonas*
1S3A	+	-	+	-	+	-	+	-	-	-	*Cellulomonas*
2S1C	+	+	-	-	-	-	+	-	+	-	*Bacillus*
3S2A	+	+	-	+	-	-	-	-	-	-	*Arthrobacter*
3S2B	+	+	-	+	+	-	-	-	-	-	*Arthrobacter*
1B1B	+	-	+	+	-	-	+	-	-	-	*Cellulomonas*
1B1H	+	+	-	-	+	-	+	-	-	-	*Pseudomonas*
2B2B	+	-	+	-	+	-	+	-	+	-	*Bacillus*
3B1B	+	-	+	-	+	-	+	-	+	-	*Bacillus*
3B1E	+	+	+	-	+	-	+	-	+	-	*Bacillus*
3B2A	+	-	+	+	-	-	+	-	-	-	*Pseudomonas*
3B2C	+	+	+	+	+	-	+	+	+	-	*Bacillus*

Note: (+) indicates a positive result; (−) indicates a negative result.

The physiological characterization outlined in **Table 3** describes the various tests used to identify the bacterial species isolated from soil samples (Mahmood et al. 2020). Each isolate was subjected to several biochemical assays, including catalase, starch casein agar (SCA), MacConkey, eosin methylene blue (EMB), mannitol salt agar (MSA), and SIM tests (sulfide, indole, and motility production), as well as methyl red and Voges-Proskauer tests. The catalase test showed positive results for all isolates, indicating their aerobic or facultative anaerobic nature. Isolates that grew on SCA and showed degradation of starch and casein, such as 1S3A and 2S1C, were likely to be *Cellulomonas* and *Bacillus* species. Selective media, such as MacConkey, EMB, and MSA, helped differentiate between gram-negative and salt-tolerant bacteria. *Pseudomonas* isolates such as 2F3A and 2F3B grew on EMB and MacConkey but not on MSA, indicating their inability to tolerate high salt concentrations. SIM tests helped assess motility and sulfur metabolism, which were positive in several *Bacillus* isolates. Indole production was limited, observed only in select isolates, such as 1S3A and 1B1B. Methyl red and Voges-Proskauer tests, used to determine fermentation pathways, yielded mostly negative results across the isolates. Collectively, the patterns of positive and negative results across these tests enabled the tentative identification of the isolates as belonging to genera such as *Arthrobacter*, *Pseudomonas*, *Cellulomonas*, and *Bacillus*.

3.4. Phenetic Dendrogram of Cellulolytic Bacteria

Fig. 2 shows the phenetic dendrogram, which illustrates the process of identifying cellulose-degrading bacteria (Flimban et al. 2019). The identification of the isolates begins with morphological characterization, which includes gram staining, cell shape, colony color, elevation, and oxygen requirements. Following this, biochemical tests were performed to differentiate further and classify the bacterial isolates, which enabled the construction of phylogenetic relationships among the isolates. The phenetic dendrogram illustrates the relationships among bacterial isolates obtained from soil samples, showing four distinct genera: *Cellulomonas*, *Arthrobacter*, *Bacillus*, and *Pseudomonas*.

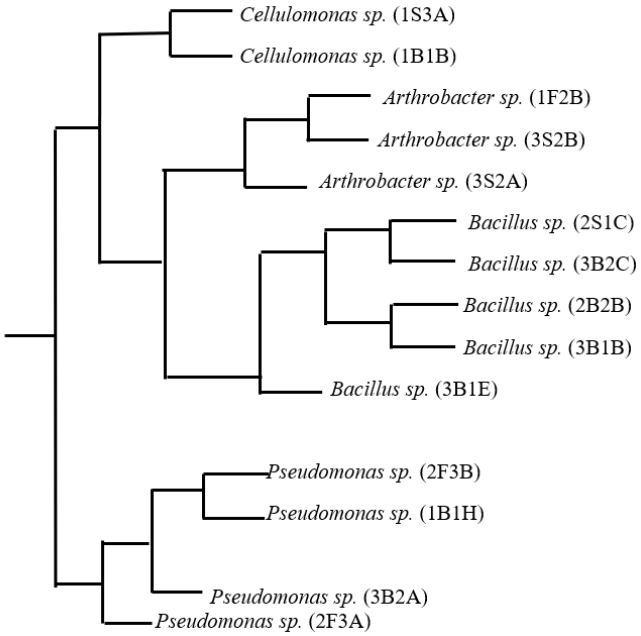


Fig. 2. Phenetic dendrogram of bacterial isolates.

The isolates belonging to the same genus clustered closely together, indicating high genetic or phenotypic similarity. *Cellulomonas* and *Arthrobacter* isolates formed tight groups, suggesting consistency across samples. The *Bacillus* group exhibited greater diversity, as indicated by the slightly distant positioning of isolate 3B1E, whereas *Pseudomonas* isolates formed two subclusters, suggesting variability within the genus. These groupings confirm the accuracy of genus-level classification and demonstrate the distribution and diversity of bacterial species across different soil sampling sites. **Fig. 3** illustrates representative examples of *Bacillus*, *Pseudomonas*, *Arthrobacter*, and *Cellulomonas*.

Isolates 1S1C, 2B2B, 3B1B, 3B2C and 3B1E have the characteristics of *Bacillus* and are identified under this genus. This is because *Bacillus* is a gram-positive, rod-shaped bacterium. Since *Bacillus* can be an anaerobe, an aerobe, and a facultative anaerobe (Bergey's Manual), it is observed in biochemical tests that isolates 1S1C, 2B2B, 3B1B, 3B2C, and 3B1E are positive in the catalase test but negative in the indole test. However, some species within this genus are known for their ability to degrade cellulose. Isolates 2F3A, 2F3B, 1B1H, and 3B2A have the characteristics of *Pseudomonas* and are identified under this genus. Bacteria of the genus *Pseudomonas* can be found in various environments, including soil, water, plants, and animal tissue. These bacteria exhibit metabolic versatility, enabling them to utilize a wide range of

substrates as nutrient sources (Rowland et al. 2018). Lists of the aerobic cellulose-degrading bacteria, which include a species from the genus of *Pseudomonas*.

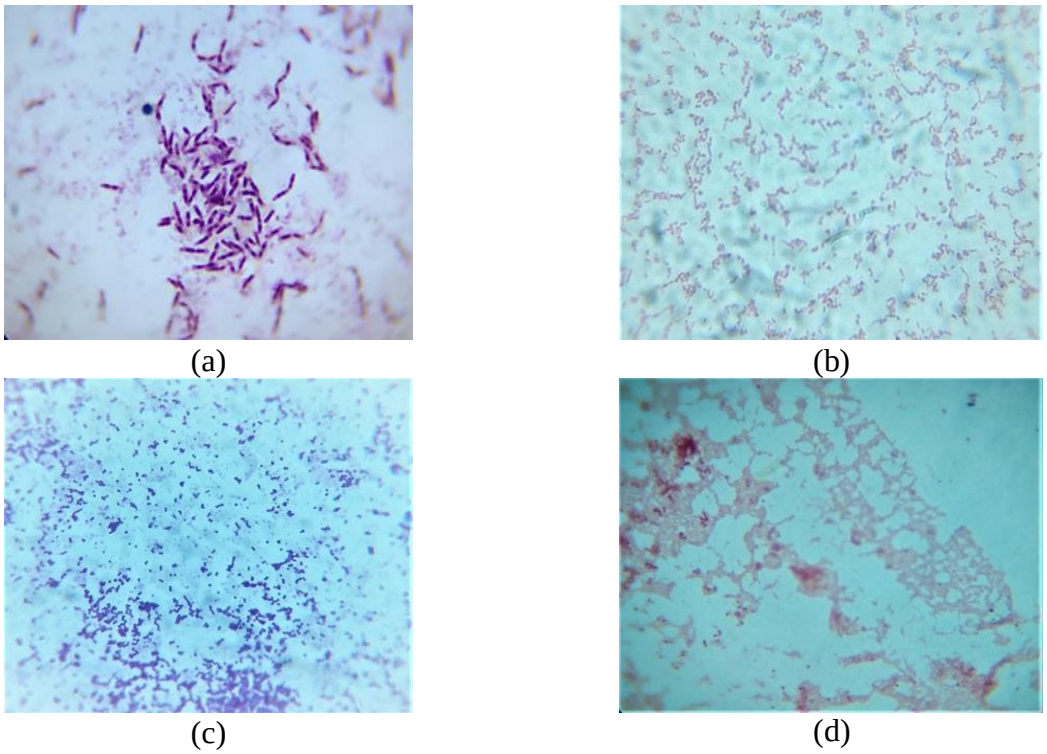


Fig. 3. Gram stain characteristic of *Bacillus* sp. from isolate: (a) 2B2B, (b) 1B1H, (c) 3S2A, and (d) 1B1B showing gram-positive reaction and rod-shaped cells.

Isolates 1S3A and 1B1B have the characteristics of *Cellulomonas* and are identified under this genus, due to their similarity in physiological characteristics. Soil appears to be the primary habitat of *Cellulomonas*, and several studies on this genus are primarily due to its cellulolytic activity. *Cellulomonas* were recovered from fresh refuse and the landfill area (Mohammadipour et al. 2021). Isolates 1F2B, 3S2A, and 3S2B have the characteristics of *Arthrobacter*. *Arthrobacter* are extremely numerous in certain soils. Fischer defined *Arthrobacter* as including all non-flagellate, rod-shaped bacteria (Bergey 1994). Only a few species under this genus can degrade cellulose.

4. Conclusions

Fourteen bacterial strains, out of seventy-six total isolates, demonstrated cellulolytic activity and were isolated from landfill sites in Barangay Santiago and Bonbonon, as well as from the forested area of Barangay Suarez in Iligan City. Based on morphological and physiological characteristics, the isolates were presumptively identified as belonging to the genera *Bacillus*, *Pseudomonas*, *Cellulomonas*, and *Arthrobacter*. Among these, *Bacillus*, *Pseudomonas*, and *Cellulomonas* are well-documented cellulose-degrading bacteria and are commonly found in soil environments. *Arthrobacter* species, although less frequently reported, have also shown limited cellulolytic potential in some studies. Results from morphological and biochemical tests indicated that *Bacillus* was the most prevalent genus, particularly in landfill environments. The identification of these cellulolytic bacteria contributes valuable information for future biotechnological applications, particularly in the conversion and utilization of biomass waste. The ability of these

isolates to utilize cellulose as a carbon source further supports the notion that landfill environments are more conducive to the growth of cellulolytic microorganisms compared to forest soils. Further studies are recommended to enhance the characterization and identification of the isolates. Additional morphological techniques, such as capsule staining and spore staining, should be conducted, along with biochemical tests including glucose fermentation, starch hydrolysis, and the use of Congo red in CMC agar to detect cellulase activity. Molecular identification using 16S rRNA gene sequencing is also highly recommended to achieve more precise taxonomic resolution at the species level.

Acknowledgments

The author sincerely thanks the Mindanao State University – Iligan Institute of Technology for allowing the researcher to conduct the study in their laboratory and For. Marne G. Origenes for making the map of the sampling site. The author also extends appreciation to Caraga State University, Department of Environmental Science, for providing technical guidance and assistance.

Author Contributions

J.M.B.S.: Conceptualization, Methodology, Data Curation, Investigation, Data Analysis, Writing – Original Draft Preparation, Writing – Review & Editing.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation

During the preparation of this work, the author used Turnitin to check plagiarism. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

References

Allen, M. E. 2005. *MacConkey Agar Plates Protocols*. American Society for Microbiology, Washington, D.C.

Arshad, M. A., Lowery, B., and Grossman, B. 1997. Physical Tests for Monitoring Soil Quality. *Methods for Assessing Soil Quality*, *Soil Science Society of America Special Publication* 49: 123–141. DOI: 10.2136/sssaspecpub49.c7

Ashraf, A., Zahra, N., Sadiqqui, F., Zeeshan, B., Sarwar, A., Ullah, Aziz, T., Alharbi, M., Alshammari, A., and Alasmari, A. F. 2024. Effect of Methylene Blue, Eosin Methylene Blue Agar, and Combinational Antibiotic on Carbapenem Resistance of *Acinetobacter baumannii* via *In Vitro and In Silico* Approach. *Applied Ecology and Environmental Research* 22(3): 2005–2026. DOI: 10.15666/aeer/2203_20052026

Bautista-Cruz, A., Aquino-Bolaños, T., Hernández-Canseco, J., and Quiñones-Aguilar, E. E. 2024. Cellulolytic Aerobic Bacteria Isolated from Agricultural and Forest Soils: An Overview. *Biology* 13(2): 102. DOI: 10.3390/biology13020102

Bergey, D. H. 1994. *Bergey's Manual of Determinative Bacteriology*. Lippincott Williams and Wilkins, Philadelphia.

Bhatia, T., Bose, D., Sharma, D., and Patel, D. 2024. A Review on Cellulose Degrading Microbes and Its Applications. *Industrial Biotechnology* 20(1): 26–39. DOI: 10.1089/ind.2023.002

Binkley, D., and Fisher, R. F. 2019. *Ecology and Management of Forest Soils*. John Wiley and Sons, Hoboken.

Bogati, K., and Walczak, M. 2022. The Impact of Drought Stress on Soil Microbial Community, Enzyme Activities, and Plants. *Agronomy* 12(1): 189. DOI: 10.3390/agronomy12010189

Butnariu, M., and Flavius, A. I. 2022. General Information about Cellulose. *Journal of Biotechnology and Bioprocess* 3(3): 1–5. DOI: 10.31579/2766-2314/077

Chang, E. H., Tian, G., and Chiu, C. Y. 2017. Soil Microbial Communities in Natural and Managed Cloud Montane Forests. *Forests* 8(2): 33. DOI: 10.3390/f8010033

Cormier, J., and Janes, M. 2014. A Double Layer Plaque Assay using Spread Plate Technique for Enumeration of Bacteriophage MS_2. *Journal of Virological Methods* 196: 86–92. DOI: 10.1016/j.jviromet.2013.10.034

Cullen, J. J., and MacIntyre, H. L. 2016. On the Use of the Serial Dilution Culture Method to Enumerate Viable Phytoplankton in Natural Communities of Plankton Subjected to Ballast Water Treatment. *Journal of Applied Phycology* 28(1): 279–298. DOI: 10.1007/s10811-015-0601-x

De Visscher, A., Haesebrouck, F., Piepers, S., Vanderhaeghen, W., Supré, K., Leroy, F., Van Coillie, E., and De Vliegher, S. 2013. Assessment of the Suitability of Mannitol Salt Agar for Growing Bovine-Associated Coagulase-Negative Staphylococci and Its Use under Field Conditions. *Research in Veterinary Science* 95(2): 347–351. DOI: 10.1016/j.rvsc.2013.05.015

Devika, C., Singhalage, I. D., and Seneviratne, G. 2021. Modification of Nutrient Agar Medium to Culture Yet-Unculturable Bacteria Living in Unsanitary Landfills. *Ceylon Journal of Science* 50(4): 505–512. DOI: 10.4038/cjs.v50i4.7949

Flimban, S., Oh, S. E., Joo, J. H., and Hussein, K. A. 2019. Characterization and Identification of Cellulose-Degrading Bacteria Isolated from a Microbial Fuel Cell Reactor. *Biotechnology and Bioprocess Engineering* 24(4): 622–631. DOI: 10.1007/s12257-019-0089-3

Harindintwali, J. D., Wang, F., Yang, W., Zhou, J., Muhoza, B., Mugabowindekwe, M., and Yu, X. 2022. Harnessing the Power of Cellulolytic Nitrogen-Fixing Bacteria for Biovalorization of Lignocellulosic Biomass. *Industrial Crops and Products* 186(12): 115235. DOI: 10.1016/j.indcrop.2022.115235

Idris, S. 2021. *Isolation and Characterization of Vancomycin Resistant Staphylococcus aureus from Wound Infections in Patients Attending Selected Hospitals in Minna, Niger State, Nigeria.* [Dissertation]. Federal University of Technology Minna. Niger State, Nigeria.

Istikorini, Y., Sari, O.Y. 2022. Identification of Endophytic Fungi of Balangeran (*Shorea balangeran* Korth.) by Morphological Characterization. *Jurnal Sylva Lestari* 10(2): 211–222. DOI: 10.23960/jsl.v10i2.547

Kaksonen, A. H. 2018. Editorial for Special Issue "Microorganisms for Environmental and Industrial Applications". *Microorganisms* 6(3): 62. DOI: 10.3390/microorganisms6030062

Khatoon, H., Solanki, P., Narayan, M., Tewari, L., Rai, J. P. N., and Khatoon, C. H. 2017. Role of Microbes in Organic Carbon Decomposition and Maintenance of Soil Ecosystem. *International Journal of Chemical Studies* 5(6): 1648–1656.

Kones, C., Mwajita, M., Kariuki, L., Kiirika, L., and Kavoo, A. 2020. Isolation and Characterization of Rhizospheric Microorganisms from Bacterial Wilt Endemic Areas in Kenya. *African Journal of Microbiology Research* 14(7): 349–360. DOI: 10.5897/ajmr2020.9292

Kozlowski, T. T., Kramer, P. J., and Pallardy, S. G. 2012. *The Physiological Ecology of Woody Plants*. Academic Press, Cambridge.

Mahmood, R., Afrin, N., Jolly, S. N., and Shilpi, R. Y. 2020. Isolation and Identification of Cellulose-Degrading Bacteria from Different Types of Samples. *World Journal of Environmental Biosciences* 9(2): 8–13.

Masngut, N., Manap, S., and Shaarani, S. 2017. Bacteria Isolation from Landfill for Production of Industrial Enzymes for Waste Degradation. *Indian Journal of Science and Technology* 10(7): 1–5. DOI: 10.17485/ijst/2017/v10i7/111224

Mor, S., and Ravindra, K. 2023. Municipal Solid Waste Landfills in Lower-and Middle-Income Countries: Environmental Impacts, Challenges and Sustainable Management Practices. *Process Safety and Environmental Protection* 174(1): 510–530. DOI: 10.1016/j.psep.2023.04.014

Mohammadipour, Z., Enayatizamir, N., Ghezelbash, G., and Moezzi, A. 2021. Bacterial Diversity and Chemical Properties of Wheat Straw-Based Compost Leachate and Screening of Cellulase Producing Bacteria. *Waste and Biomass Valorization* 12(6): 1293–1302. DOI: 10.1007/s12649-020-01119-w

Mostaghimi, K., and Behnamian, J. 2023. Waste Minimization Towards Waste Management and Cleaner Production Strategies: A Literature Review. *Environment, Development and Sustainability* 25(11): 12119–12166. DOI: 10.1007/s10668-022-02599-7

Nasrollahzadeh, M., Sajjadi, M., Nezafat, Z., and Shafiei, N. 2021. *Polysaccharide Biopolymer Chemistry*. Elsevier, Amsterdam.

Nonaka, K., Todaka, N., Ōmura, S., and Masuma, R. 2014. Combination Cellulose Plate (Non-Agar Solid Support) and Agar Plate Method Improves Isolation of Fungi from Soil. *The Journal of Antibiotics* 67(11): 755–761. DOI: 10.1038/ja.2014.65

Nosratzehi, T., Nosratzehi, M., Nosratzehi, S., and Lotfi, F. 2019. The Comparison of the Effect of Curcumin with Nystatin on Inhibition Level of *Candida albicans*. *Journal of Experimental Pharmacology* 11: 93–97. DOI: 10.2147/jep.s215843

Olee, A. 2016. *Isolation and Identification of Pathogenic Bacteria from College Premises.* [Dissertation]. St. Xavier's College. Kolkata, West Bengal, India.

Padhan, K., Patra, R. K., Sethi, D., Panda, N., Sahoo, S. K., Pattanayak, S. K., and Senapati, A. K. 2023. Isolation, Characterization and Identification of Cellulose-Degrading Bacteria for Composting of Agro-Wastes. *Biomass Conversion and Biorefinery* 15(4): 4925–4939. DOI: 10.1007/s13399-023-04087-y

Paray, A. A., Singh, M., Mir, M. A., and Kaur, A. 2023. Gram Staining: A Brief Review. *International Journal of Research and Review* 10(9): 336–341. DOI: 10.52403/ijrr.20230934

Patra, J. K., Das, G., Das, S. K., and Thatoi, H. 2020. *A Practical Guide to Environmental Biotechnology*. Springer, Singapore. DOI: 10.1007/978-981-15-6252-5_4

Pradeep, N. S., and Edison, L. K. 2022. *Microbial Beta Glucanases: Molecular Structure, Functions and Applications*. Springer Nature, Singapore.

Rahmadwiati, R., Wicaksono, R.L., and Apriyanto, D. 2022. Estimation and Economic Valuation of Above-Ground Carbon in Forest Area with the Special Purpose of Gunung Bromo, Karanganyar, Central Java, Indonesia. *Jurnal Sylva Lestari* 10(1): 116–126. DOI: 10.23960/jsl.v10i1.548

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., and Tuohy, K. 2018. Gut Microbiota Functions: Metabolism of Nutrients and Other Food Components. *European Journal of Nutrition* 57(1): 1–24. DOI: 10.1007/s00394-017-1445-8

Sakhno, N. G., Gunar, O. V., Roshchina, M. V., Kolosova, L. V., and Grigor'eva, V. É. 2018. Isolation of Anaerobic Bacteria during Quality Analysis of Medicinal Products. *Pharmaceutical Chemistry Journal* 52(6): 569–572. DOI: 10.1007/s11094-018-1861-y

Sousa, F., Nascimento, C., Ferreira, D., Reis, S., and Costa, P. 2023. Reviving the Interest in the Versatile Drug Nystatin: A Multitude of Strategies to Increase Its Potential as an Effective and Safe Antifungal Agent. *Advanced Drug Delivery Reviews* 199: 114969. DOI: 10.1016/j.addr.2023.114969

Sundarraj, A. A., and Ranganathan, T. V. 2018. A Review on Cellulose and Its Utilization from Agro-Industrial Waste. *Drug Invention Today* 10(1): 89–94.

Thomas, P., Sekhar, A. C., Upreti, R., Mujawar, M. M., and Pasha, S. S. 2015. Optimization of Single Plate-Serial Dilution Spotting (SP-SDS) with Sample Anchoring for Bacterial and Yeast CFU Enumeration and Single Colony Isolation from Diverse Samples. *Biotechnology Reports* 8: 45–55. DOI: 10.1016/j.btre.2015.08.003c

University of Illinois Extension. 2018. *The Science of Composting*. <https://web.extension.illinois.edu/homecompost/science.cfm> (March 01, 2019)

Valavanidis, A. 2023. Global Municipal Solid Waste (MSW) in Crisis. *Two Billion Tonnes of MSW Every Year, a Worrying Worldwide Environmental Problem* 1: 1–28.

Virtual Amrita Laboratories Universalizing Education. 2018. *Isolation and Identification of Two Bacteria Unknowns.* Amrita Vishwa Vidyapeetham. <http://vlab.amrita.edu/?sub=3&brch=76&sim=1109&cnt=1> (March 01, 2019)

Yamakawa, C. K., Qin, F., and Mussatto, S. I. 2018. Advances and Opportunities in Biomass Conversion Technologies and Biorefineries for The Development of a Bio-Based Economy. *Biomass and Bioenergy* 119: 54–60. DOI: 10.1016/j.biombioe.2018.09.007