# ISOLATION, SCREENING AND IDENTIFICATION OF CELLULOLYTIC BACTERIA FROM WOOD INDUSTRY AND BAMBOO TREES SOIL SAMPLES

Nan Nan Htwe[1], Nyein Nyein Hlaing[2], Aye Aye Khaine[3], Phyo Wai Khing[4]

## Abstract

Cellulases are key enzymes that hydrolyze cellulose into simpler sugars, making them vital for applications such as bioethanol production and use in the textile and paper industries. Bacteria obtained from different soil samples in South Dagon Township, Yangon Region were assessed for cellulose degradation. Cellulose degrading bacteria were isolated from wood industry soil and bamboo tree soil samples using serial dilution and pour plate method. This study was directed towards the isolation and screening of cellulase producing bacteria from wood industry soil and bamboo tree soil samples on CMC (carboxymethyl cellulose) agar plates, by Congo Red staining. A total 18 bacteria were isolated from wood industry soil and bamboo tree soil. Out of 18 isolated bacteria, six isolates were showed clear zones of hydrolysis on CMC agar. Among cellulolytic bacteria, two cellulolytic bacteria were isolated from wood industry soil and four cellulolytic bacteria were isolated from bamboo tree soil. Among the six strains, *Bacillus* spp.2 was showed the highest cellulose hydrolysis activity from bamboo tree soil. Bacteria identification was carried out according to colony morphology, gram stain appearance and their biochemical reaction based on Bergey's Manual of Determinative Bacteriology. These findings highlight the potential of local soil environments as a source for highly active, industrial-relevant cellulolytic bacterial strains.

**Keyword**: wood industry soil and bamboo tree soil, isolation, screening and identification, cellulolytic bacterial strains

## Introduction

Bacteria are the major cellulose-producing microbes involved in the degradation process. Cellulolytic bacteria have been isolated from a wide diversity of environments such as soils, composts and decaying plant litter, which play a specific role in the biodegradation of organic materials during decomposition (Medie *et al*., 2012). Soil is the richest ecosystem which is the most imperative source for the presence of hydrolytic enzyme-producing microbes such as bacteria, fungi, actinomycetes etc. The microorganisms in the soil produce different hydrolytic enzymes such as amylases, proteases, cellulases, lipases etc. There are several reports available on the production of cellulases from microbes (Bhagat *and Kokitkar*., 2021).

Moreover, they play a pivotal role in plant residue decomposition since microorganisms are the main producer of enzymes that participate in the degradation processes of plant cell wall polymers, including cellulose, hemicellulose and lignin in soils (Lopez-Mondejar *et al.*, 2016). Cellulose is an abundant organic compound present on earth. Each cellulose molecule contains 10,000 glucose units (Daba and Krishna, 2019). Cellulose is the most common organic polymer. Cellulose composes a liner polymer of glucose. Cellulose need biodegradation before using an energy source of glucose. Biodegradation of cellulose using cellulolytic bacteria can be an effective way of serving low price of high-quality feed. Cellulolytic bacteria synthesize a set of enzymes capable of breaking down cellulose by hydrolyzing the glycosides β-1.4 polymer

[1]Department of Biology, Yangon University of Education
[2] Department of Zoology, Dagon University
[3] Department of Zoology, University of Yangon
[4] Department of Biology, Yangon University of Education

cellulose bond (Whistler and Smart, 1953). Fermentation with cellulolytic bacteria can increase the sugar and crude protein levels, degrade cellulose and crude fiber content (Ardiansyah and Aspet, 2019).

Wood industry soil is a rich source for obtaining cellulose degrading bacteria because of abundant diverse group of cellulolytic microorganisms owing to the presence of cellulose in wood works and timber. Further, its wide availability, ease of collection and cost effectiveness also plays important role for its selection (Tolulop *et al.*, 2019).

Cellulose, the largest component of plant residues- terrestrial ecosystem and huge source of energy for microorganism, indirectly responsible for soil organic matter decomposition. Degradation processes of plant cell wall polymers, including cellulose, hemicellulose and lignin in soils (Arman *et al*., 2022). There is huge demand for cellulase enzyme in many industries like textile and paper production factories. (Daba and Krishna, 2019). To improve the quality of nutrients and digestibility of feed ingredients at a cheaper price than the use of commercial cellulase enzymes (Wenny *et al*., 2017). The objective of this study are as follow,

- To investigate the cellulose degrading activity of bacteria from wood industry and bamboo tree soil
- To determine the cellulolytic activity from isolated bacteria
- To identify the isolated cellulolytic bacteria

## Materials and Methods

### Study area period

Soil samples were taken from wood industry soil and bamboo trees soil in South Dagon Township, Yangon Region. The samples were carried out the Microbiology Laboratory, Department of Zoology, Dagon University. The study period lasted from November, 2023 to May, 2024.

### Materials

The apparatus and equipment used for the laboratory work were water distiller, analytical balance, biosafety cabinet, vortex mixture, stirrer hotplate, autoclave, refrigerator, hot air oven, incubator, colony counter, various kinds of glassware, pipettes, microscope glass slides, inoculation nichrome wire loop, aluminium foil, masks, glove, long coat and sterilized cotton.

The culture media and biochemical test media used in bacteriology study consisted Plate Count Agar, Nutrient agar, Carboxy Methyl Cellulose agar, Gram staining kits, Triple Sugar Iron (TSI) agar, Simmon's Citrate agar, Methyl-Red Voges-Prokauer (MR VP) broth, Urea agar, Sulphide Indole Motility (SIM) medium, Geltinase Enzyme, Oxidase reagent, Starch Hydrolysis test, Salt tolerance test and Sugar fermentation test etc. Methyl Red reagent, α-naphthol reagent, 40% KOH solution containing 0.3% creatine, 3% $H_2O_2$ and Kovac's reagent.

### Methods

### Sample collection

Soil samples were collected from the wood industry and bamboo tree soil in South Dagon Township, Yangon Region. Each soil sample (100g) was randomly collected from the superficial

soil (depth 1 to 10 cm) aseptically and stored in sterile plastic bags. After labeled and transported to the Microbiology Laboratory of Zoology Department, Dagon University.

**Preparation of samples**

Wood industry soil was designed to WS and bamboo soil was designed to BS. One gram of each soil samples were serially diluted with 9mL of distilled water by using a vortex mixer.

**Serial dilution**

One gram of collected soil sample was serially diluted in 10 mL of sterile distilled water up $10^{-1}$ to $10^{-10}$. 1 g of each dilution ($10^{-3}$ to $10^{-10}$) was inoculated on plate count agar using pour plate method and incubated at $37^0$ C for 24 hours. Subsequently, the number of growing colonies on each plate were counted for estimation of the number of total viable bacteria. The colony numbers only between 30 to 300 in each plate were used to calculate the colony forming unit (CFU/g-1). Then the number of colony were multiplied with the dilution factor and the bacteria counts in CFU/ g-1 were calculated (Dubey and Maheshawri, 2002).

**Morphological characterization of the bacterial isolates**

The bacteria isolates were isolated and recorded the colony morphology such as, color, size, shape, surface, elevation, margin and texture, were recorded. (Bisen and Verma, 1998).

**Determination of carboxy methyl cellulase (CMC) activity of bacteria isolates**

Pure cultures of bacterial isolates were individually transferred in Carboxy Methyl Cellulose (CMC) agar plates. After incubation for 48 hrs, CMC agar plates was flooded with 1 % Congo red and allowed to stand for 15 mins at room temperature. One molar NaCl was thoroughly added and used for counterstaining of the plates. Clear zones appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the largest clear zone were selected for identification and cellulase production in submerged system (Andro *et al*., 2004).

**Morphological identification - Gram staining procedure**

A pure colony from culture plate was picked up and smears on clean glass slide, air-dried, heat-fixed and stained with crystal violet for one minute. The slide was washed in a gentle and indirect stream of tap water. The slide was flooded with the mordant: Gram's iodine for one minute and washed again. The slide was decolorized with acetone for 30 seconds or added drop by drop to slide until decolorizing agent running from the slide runs clear and washed out. Lastly, the slide was flooded with counterstain, safranin for 30 seconds to one minute and washed out. The gram stained smear was examined under oil immersion field of Brightfield microscope. Gram-negative bacteria will be stained pink or red and gram-positive bacteria will be stained blue or purple.

**Biochemical characterization of the cellulolytic bacteria**

All isolates were subjected to the following standard biochemical tests described using dehydrated media. These tests included (i) Simon’s citrate utilization test, (ii) urea test, (iii) gelatinase test, (iv) sulfur indole motility (SIM) test, (v) triple sugar iron (TSI), (vi) Oxidase test, (vii) catalase test, (viii) starch hydrolysis test, (ix) sodium chloride test and (x) glucose fermentation test. The characteristic features of isolated bacteria like colony and cell morphology, gram staining nature and biochemical properties obtained in the present work were

compared to the standard described in Bergey's Manual for Determinative Bacteriology (Breed *et al*., 1994); Cowan and Steel's Manual for Identification of Medicine Bacteria (Cowan, 1975).

## Results

**Bacteria counts of the wood industry and bamboo tree soil samples**

The total viable count of bacteria on plate count agar of soil sample from wood industry soil was 3.92 x $10^8$ CFU/ g-1 and soil sample from bamboo tree soil was (2.27 x $10^7$ CFU/ g-1) as shown in (Table 1).

**Cellulolytic activity and cell morphology of the isolated bacteria**

In the present study, a total of 18 bacteria were isolated from wood industry and bamboo tree soil samples. All cellulolytic bacteria were designated into SI.B and SI.F from wood industry soil and SII.D, SII.E, SII.F and SII.J from bamboo tree soil. Out of these 18 isolates, 6 isolates showed a distinct clear zone around colonies in CMC agar plate after congo red staining as shown in (Plate.1). Among all cellulolytic bacteria, SII.D exhibited the highest cellulose-degrading activity range of 38 mm, while SI.F was the lowest cellulose-degrading activity range of 10 mm as shown in (Table 2).

After gram staining one isolate from SI.B was gram positive, rod shape arrange in chain and five isolates (SI.F, SII.D, SII.E, SII.F, SII.J) were gram positive, rod shape bacteria as shown in (Plate.2). The biochemical characteristics of cellulolytic bacteria were designated into two genera; *Streptobacillus* sp. and *Bacillus* spp. was identified according to Bergey's Manual for Determinative Bacteriology (Breed *et al*., 1994); Cowan and Steel's Manual for Identification of Medicine Bacteria (Cowan, 1975) as shown in (Table 4). In wood industry soil, both *Streptobacillus* sp. and *Bacillus* sp. were found and *Bacillus* spp.2, *Bacillus* spp.3, *Bacillus* spp.4 and *Bacillus* spp.5 were isolated only in bamboo tree soil.

**Table (1) Colony forming unit of cellulolytic bacteria from wood Industry soil and bamboo tree soil samples**

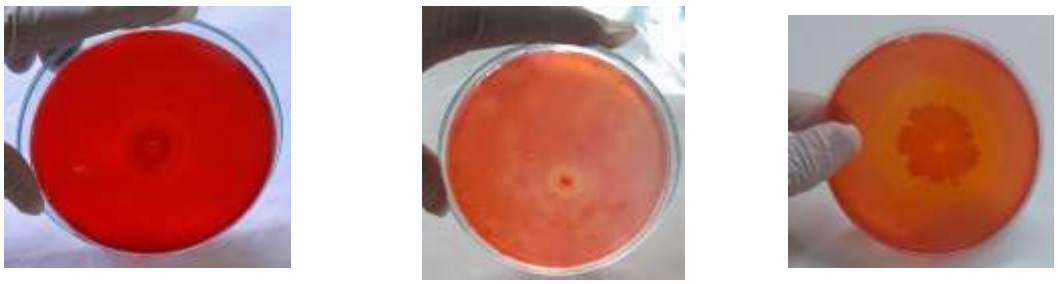
| Sr. no. | Sample code | First Count CFU/g | Duplicate Count CFU/g | Total CFU/g | Average Count CFU/g |
|---|---|---|---|---|---|
| 1 | WS | 7.11 x $10^8$ | 7.28 x $10^8$ | 7.84 x $10^8$ | 3.92 x $10^8$ |
| 2 | BS | 2.30 x $10^7$ | 2.24 x $10^7$ | 4.54 x $10^7$ | 2.27 x $10^7$ |

WS = wood industry soil, BS = bamboo tree soil

Table (2) Evaluation of highly potential cellulolytic bacterial isolate in CMC agar medium

| Sr.no. | Bacteria isolates code | Zone diameter (mm) |
|---|---|---|
| 1. | SI. B | 28 mm |
| 2. | SI. F | 10 mm |
| 3. | SII. D | 38 mm |
| 4. | SII. E | 27 mm |
| 5. | SII. F | 12 mm |
| 6. | SII. J | 36 mm |

A. SI. B B. SI. F C. SII. D



D. SII.E E. SII. F F. SII. J

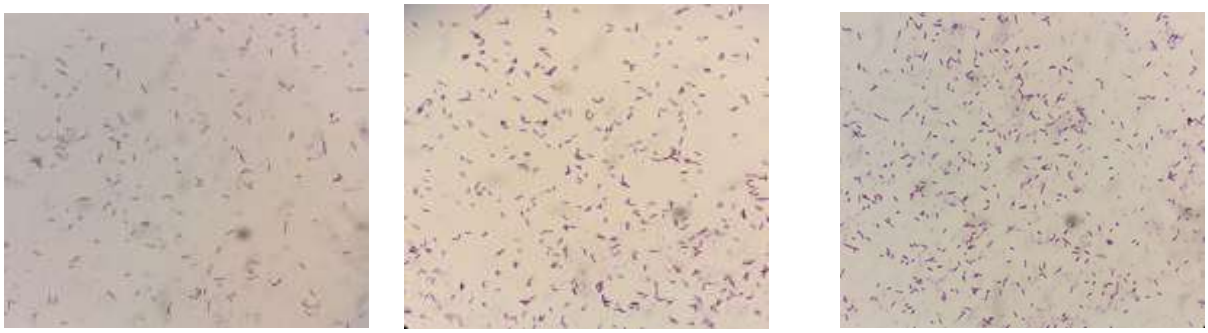
**Plate 1.** Cellulolytic activity of isolated bacteria from wood industry and bamboo tree soil samples on CMC agar for two days old culture

Table (3) Cell morphology and gram staining reaction of cellulolytic bacteria six isolates

| No | Isolates strains | Shape | Margin | Elevation | Size | Texture | Appearance | Colony color | Shape | Gram-staining | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | SI.B | Circular | Entire | Flat | Large | rough | glistening | White | rod | + | Motile |
| 2. | SI.F | Circular | Entire | Convex | Large | smooth | dull | Cream | rod | + | Motile |
| 3. | SII.D | Circular | Entire | Convex | Large | rough | dull | Cream | rod | + | Motile |
| 4. | SII.E | Circular | Entire | Convex | Large | rough | dull | Cream | rod | + | Motile |
| 5. | SII.F | Circular | Entire | Convex | Large | rough | glistening | Cream | rod | + | Motile |
| 6. | SII.J | Circular | Entire | Convex | Large | rough | dull | Cream | rod | + | Motile |

+ = positive, - = negative

A.SI. B B.SI. F C.SII.D



D. SII.E E. SII. F F. SII. J

Plate 2. Gram staining appearance of cellulolytic bacteria from wood industry soil and bamboo tree soil

**Table (4) Biochemical characteristics of cellulolytic bacteria isolates from wood industry soil and bamboo trees soil**

| Sr. no | Isolate code | SC | Urea | TSI | | | VP | Gel | SIM | | | Oxi | Cata | Star | Salt tolerance | Glu | Tentative genera |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | S | B | Gas | | | S | I | M | | | | | | |
| 1. | SI. B | + | + | K | A | - | + | - | - | - | + | - | + | + | + | + | *Streptobacillus* sp. |
| 2. | SI. F | - | + | K | K | - | - | + | - | - | + | + | + | - | + | + | *Bacillus* spp.1 |
| 3. | SII. D | + | + | K | A | - | + | - | - | - | + | + | + | - | + | + | *Bacillus* spp.2 |
| 4. | SII. E | + | + | K | A | - | - | + | - | - | + | + | + | - | + | + | *Bacillus* spp.3 |
| 5. | SII. F | - | + | K | A | - | - | - | - | - | + | + | + | - | + | + | *Bacillus* spp.4 |
| 6. | SII. J | - | + | K | A | - | - | - | - | - | + | + | + | + | + | + | *Bacillus* spp.5 |

(+) = positive reaction, (-) = negative reaction, No gas
S = slant, B = butt, G= gas, SC=Simmon Citrate Utilization, MR = Methyl Red, VP = Voges Proskauer,
Gel = Gelatinase, Urea = urea test, SIM = Sulphide Indole Motility, Ca = catalase, A= acid, K= alkaline,
ST= Salt Tolerance test, Glu = Glucose concentration test, Cata = Catalase test, Star = Starch Hydrolysis test.

**Table (5) Summary of isolated bacteria from wood industry soil and bamboo trees soil samples**

| No. | Tentative genera of species | WS | BS |
|---|---|---|---|
| 1 | *Streptobacillus* sp. | 1 | - |
| 2 | *Bacillus* spp. | 1 | 4 |
| 3 | Unidentified specie | 5 | 7 |
| 4 | Total isolates | 7 | 11 |

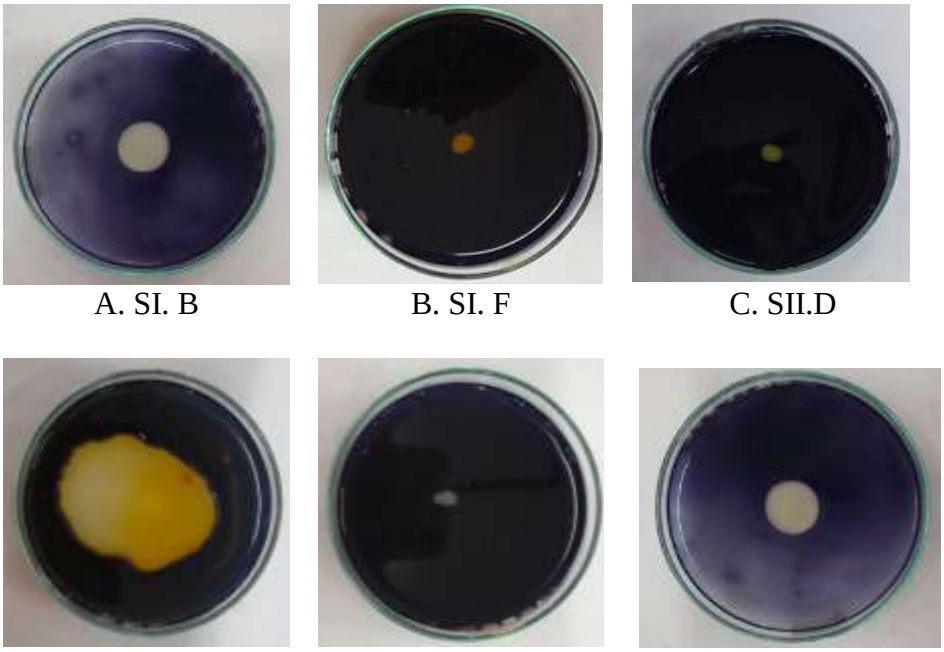
WS = Wood industry soil, BS=Bamboo tree soi

A. S.I B B. S.I F C. S.I B and S.I F for salt tolerance test D. S.I B and S.I F for glucose test

**Plate 3.** Biochemical tests from wood industry soil

A. S.II D B. S.II E C. S.II F D. S.II J

SC=Simmon Citrate Utilization, Urea = urea test, MR = Methyl Red, VP = Voges Proskauer, Gel = Gelatinase, SIM = Sulphide Indole Motility,
Plate 4. Biochemical tests from bamboo tree soils



A. SI. B B. SI. F C. SII.D

D. SII. E E. SII. F E. SII. J

**Plate 5.** Starch hydrolysis test from wood industry soil and bamboo tree soil

**Plate 6.** Salt tolerance test for S.II D, S. II E, S. II F and S. II J

**Plate 7**. Sugar fermentation test (glucose) for S.II D, S. II E, S. II F and S. II J


**Plate 8.** Catalase test from wood industry soil and bamboo tree soil

A. SI. B B. SI. F

**Plate 9.** Oxidase test form wood industry tree soil

A. SII. D B. SII. E C. SII. F D. SII. J

**Plate 10**. Oxidase test form bamboo tree soil

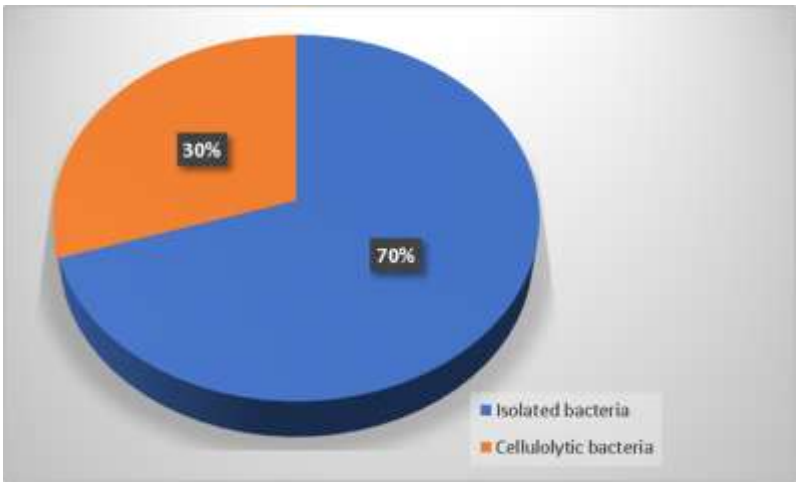
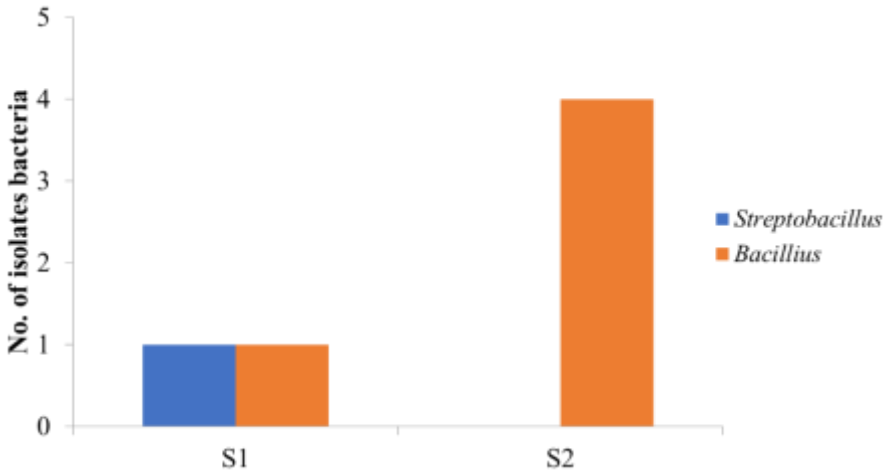



**Figure. 1** Percentage of cellulolytic bacteria isolated from the wood industry soil and bamboo tree soil**.**




SI = wood industry soil, S II = bamboo tree soil

**Figure.2** Number of cellulolytic bacteria isolated from the wood industry soil and bamboo tree soil samples

## Discussion

A total of 18 isolates were obtained from wood industry soil and bamboo tree soil samples. Out of 18 isolates, 6 isolates were produced cellulose enzymes and showed cellulolytic activity on CMC agar. The isolated cellulolytic bacteria were identified as *Streptobacillus* spp. and *Bacillus* spp. according to their colony morphologies, gram staining and biochemical characteristics. In the present study, the total viable count (CFU/ g-1) of cellulolytic bacteria in the analyzed soil samples were ranged from (2.27 x $10^{-7}$ CFU/ g-1) to (3.92 x $10^{-8}$ CFU/ g-1) which indicated a significant population of isolated bacteria. Yi Lwin Kyaw Zaw, (2013) in Yangon, cultivated soils from Garbage and Garbage dump showed higher bacterial population than present study which were ranged from (1.3 x $10^{-9}$ CFU/g) and (6.0 x $10^{-8}$ CFU/g). It was possible due to soils sample taken from different areas with different microbial community composition. Khotimah *et al*., (2020) from Indonesia also found the total bacteria count of peat forests soil were (2.1×$10^{3}$ CFU/ g-1) to (5.9×$10^{4}$ CFU/ g-1) which was lower than the present study. The different in total viable count of bacteria compared with above findings might be due to different locations, climate and temperature of soils.

In the current study, out of 18 bacterial isolates, 6 (30%) isolates showed cellulose degrading activity. Misbah *et al*., (2020) in Pakistan reported among 24 isolates from different soils, 6 strains (40%) showed cellulase production. Riad *et al*., (2020) in Bangladesh isolated 42 bacterial isolates from various soil samples (sawdust). Among then, 24 (57%) isolates showed cellulolytic activity with a distinct clear zone. The different percentage of cellulolytic activity of bacterial isolates in compared with above studies may be due to different soils that may contain substrates with higher proportion of cellulose and the existence of microenvironments where different growth conditions for cellulose degrading bacteria are present.

Among six cellulolytic bacteria, the maximum clear zone diameter of cellulolytic bacteria was about 38 mm (SII.D), while the minimum was 10 mm (SI.F). These results showed higher than the findings observed by Hatami *et al*., (2008) in Iran who reported the hydrolysis zone diameter of cellulose-degrading aerobic bacterial isolates from farming and forest soil were 0.15 cm to 1.37 cm. However, Mohammed *et al*., (2017) in Egypt who observed 42 mm to 23 mm of the clear zone diameter from garden soil. The zone diameter in present study was lower than of their findings.

In this study, among six isolated cellulolytic bacteria, 5 isolates were identified as *Bacillus* spp. and one isolate was identified as *Streptobacillus* spp. These results are in agreement with those reported in previous studies where the cellulose degrading bacteria have been identified and isolated from different environments like soil. Rastogi *et al*. (2009) also observed *Bacillus* spp. in soil. This finding was consistent with Abdel-Aziz *et al*., (2021) in Egypt who also identified *Bacillus cereus, Bacillus licheniformis* from farm fields soils. Another study reported by Misbah *et al*., 2020 in Pakistan also identified predominance of *Bacillus spp.* such as *Bacillus megaterium, Bacillus aerius, Bacillus paralichniformis* and *Bacillus flexus* from old paper, and old wood soil.

Moreover, Yi Lwin Kyaw Zaw (2013) in Yangon, also isolated *Bacillus subtilis and Bacillus cereus* from cultivated soil and Garbage dump soils. Vimal, (2016) in Kerala, were mentioned *Bacillus subtilis* and *Bacillus cereus* from industrial and agricultural soil. Shweta and Seema, (2021) in India, were identified *Bacillus subtilis, Bacillus flexus, Bacillus licheniformis* and *Bacillus paralicheniformis* from garden soil and forest soil. The different study sites and the current study sites of cellulolytic bacterial isolates in compared with above studies may be due to differences in sampling sites that may contain substrates with higher proportions of cellulose. Additionally, the present of unit microenvironments may offer distinct growth condition that favor specific cellulose-degrading bacteria.

## Conclusion

Six highly potent cellulolytic bacterial strains were successfully isolated and screened from wood industry and bamboo tree soil samples in South Dagon Township. These strains were tentatively identified as *Bacillus* spp. and *Streptobacillus* sp., with *Bacillus* being the dominant and most active genus. The isolate *Bacillus* spp. 2 (SII.D) from bamboo tree soil demonstrated the highest cellulolytic activity (38 mm clear zone), warranting further research into its enzyme characteristics for biotechnological utilization.

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