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Screening and Evaluation of Potential Cellulose Degrading Bacteria from Food Wastes of Wolkite University Student's Cafeteria

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Abstract

Food waste, which includes unwanted portions of raw or cooked food discarded during or after preparation, is a significant contributor to environmental pollution. This waste is rich in cellulose, an organic compound. This study aimed to screen and evaluate cellulose-degrading bacteria from waste foods. Bacterial isolates were cultured on a carboxymethylcellulose screening agar medium for this purpose. These isolates were further characterized based on their morphological and biochemical properties and were optimized for growth conditions, including pH, temperature, and heavy metal tolerance. From the initial screening, 30 bacterial isolates were obtained, of which 10 demonstrated cellulolytic activity. Among these, 4 isolates exhibited the highest cellulolytic activity, as determined by their cellulolytic index values. All 4 isolates were found to be gram-negative, non-motile, and tested negative for the oxidase, catalase, and Voges-Proskauer (VP) tests. However, they tested positive for the methyl red (MR) test, urease test, and citrate utilization test. Optimal growth was observed at a neutral pH of 7 and a temperature of 35 °C. Additionally, these isolates showed significant tolerance to mercury in the heavy metal tolerance assay, withstanding concentrations between 0.01% and 0.02%. These findings offer a valuable foundation for identifying and evaluating potential cellulolytic bacteria, which could be utilized in bioethanol production and other industrial applications. Further research is needed to carry out the molecular characterization of these cellulose-degrading bacteria at the species level.

Keywords: Food Waste, Cellulose, Cellulose-Degrading Bacteria, Carboxy-Methylcellulose

1. Introduction

Food waste, which consists of discarded portions of raw or cooked food, is a significant contributor to environmental pollution [1]. It is no longer suitable for consumption and constitutes a large portion of household waste [2]. Approximately 50% of municipal waste is composed of organic material, including food waste. With much of this waste being deposited in landfills, a significant amount of potential organic material is lost [2]. The Food and Agriculture Organization of the United Nations recently reported that about 50% of food globally is wasted before and after it reaches consumers, amounting to 1.3 billion tons of food waste annually. This extensive disposal of food waste poses serious challenges in waste management and pollution control, as it is not easily biodegradable [3]. Cellulose, a major structural component of various food wastes, represents a renewable and abundant source of energy. This cellulosic biomass has significant potential for conversion into various value-added bioproducts [4]. Cellulose is characterized by its crystalline structure, composed of long chains of β 1-4 linked glucose units [2].

Interest in food waste prevention and recovery programs has gained significant global attention. This is driven by factors such as resource conservation, economic considerations, and the environmental impact of food waste [5]. Research has been conducted for many years on the isolation and characterization of cellulose-degrading bacteria from a wide range of sources, including soil, municipal waste, ruminant feces, compost, and various ecological niches [6]. These bacteria produce an extracellular enzyme known as cellulase, which is crucial for breaking down cellulose.

Studies have identified that cellulase is produced by various bacteria from the genera Bacillus, Cytophaga, Cellvibrio, Cellulomonas, Pseudomonas, and Micrococcus [7]. These bacteria play a vital role in the biosphere by breaking down complex polymer cellulose into valuable products such as monomer sugars, microbial biomass proteins, compost, antibiotics, and other materials essential for human use [1].

A wide range of microorganisms, including bacteria, fungi, protozoa, and viruses, can be found in food waste [1]. These microorganisms utilize food waste as a source of nutrients, breaking it down into simpler molecules through metabolic processes. Food waste contains various macromolecules, and microorganisms secrete extracellular enzymes to degrade these molecules, making them more accessible for use. Microorganisms capable of hydrolyzing cellulose molecules are referred to as cellulolytic microorganisms [2]. Among these diverse microorganisms, bacteria are the primary agents responsible for cellulose degradation. Bacteria have a faster growth rate compared to fungi, making them highly suitable for industrial applications [9]. Bacteria are ubiquitous, thriving in a variety of ecological environments. They are considered extremely valuable due to their applications in biofuel production, the paper and textile industries, fermentation processes such as brewing, baking, cheese, and butter production, chemical manufacturing, and numerous other biological activities [2].

The isolation of cellulose-degrading bacteria has been reported in various parts of the world, but in Ethiopia, this area of research is still in its early stages. Therefore, screening and evaluating of cellulose-degrading bacteria could have significant applications across different industries in Ethiopia. This study was specifically designed to isolate and assess potential cellulose-degrading bacteria from food waste.

2. Materials and Methods

2.1 Description of the Study Area

The study was conducted at Wolkite University Molecular Biotechnology laboratory. Wolkite University is situated at a distance of 337 km from Hawassa and 158 km away from south west of Addis Ababa.

2.2 Sample Collection

Four food waste samples were collected from the disposal site of a student cafeteria. Pre-sterilized screw-cap glass vials and bottles were used for sample collection. Using a sterile spatula, waste patches weighing approximately 2 g were scraped from four different spots, each about 2 m apart, and placed into separate vials. The samples were collected simultaneously and labeled as follows: S1 for the sample taken from the outer soil layer where food waste was dumped, S2 for the sample taken from underground by scraping waste, S3 for the sample from potato, onion, and various peels of unprocessed food waste, and S4 for the sample from a mixture of different types of waste. Each sample was placed in a clean, sterile sample bottle, sealed, and transported to the biotechnology laboratory for the isolation of cellulose-degrading bacteria.

2.3 Isolation of Cellulose Degrading Bacteria

Serial dilution techniques were employed for the isolation of bacteria [1]. In this method, a sample suspension was prepared by adding 1 g of soil mixed with waste to 10 ml of sterile water, followed by vigorous shaking for at least 1 minute. The suspension was then allowed to settle for a short period. Sterile dilution blanks were labeled sequentially, starting from the stock solution and ranging from 10^{-1} to 10^{-7}. From each diluted sample suspension, 0.1 ml was spread onto CMC agar plates and incubated at 30°C for 48 hours, with the pH adjusted to 7 at room temperature.

2.4 Screening of Cellulose Degrading Bacterial Isolates

Screening for cellulose-degrading bacteria was conducted following the methods of Kaur and Arora [1]. Pure colonies of bacterial isolates were individually transferred to minimal agar medium supplemented with 1% CMC (carboxymethyl cellulose). After 48 hours of incubation, the CMC agar plates were flooded with 1% Congo red solution and left to stand for 15 minutes at room temperature. The plates were then thoroughly washed with 1M NaCl. Clear zones appeared around the bacterial colonies, indicating cellulose hydrolysis. The size of both the colonies and the clear zones around them were measured in centimeters to calculate their cellulolytic index, which was used to identify the bacterial isolates with the highest cellulolytic activity.

2.5 Characterization of Cellulolytic Bacteria

Identification and characterization of cellulolytic bacteria were performed based on their morphological characteristics, including Gram staining (using the KOH method) and motility tests, as well as biochemical tests such as the urease test, oxidase test, MR-VP test, catalase test, starch hydrolysis test, and citrate utilization test [10].

2.6 Determination of Optimal pH, Temperature and Heavy Metal Tolerance Assay for CDB

To determine the optimal growth conditions for each bacterial isolate, different temperatures (30°C, 35°C, 40°C, 45°C, 50°C), pH levels (5.0, 6.0, 7.0, 8.0), and heavy metal concentrations (0.01%, 0.02%, 0.03%, 0.04%, and 0.05% w/v) were studied. The medium was prepared in test tubes and inoculated with each bacterial isolate. The test tubes were then incubated at 37±2°C for 48 hours. After the incubation period, the growth of the cultures was measured three times using a UV spectrophotometer at 560 nm [1].

2.7 Statistical Analysis

One-way analysis of variance (ANOVA) was performed using the Statistical Package for the Social Sciences (SPSS, version 20) to determine significant differences under various conditions. Three replicates of measurements were taken for each condition. A significant difference was considered to be present when $p < 0.05$.

3. Results and Discussions

3.1 Isolation of Cellulose Degrading Bacteria

A total of 30 bacterial isolates were obtained from four different food waste samples, as detailed in Table 1. Specifically, there were 11 isolates from sample one (S1), 6 isolates from sample two (S2), 6 isolates from sample three (S3), and 7 isolates from sample four (S4). These findings are consistent with previous studies, which have reported the isolation of cellulolytic microorganisms from various environments [11].

No	Sample site	Number of samples	Isolate number	Labeled as
1	From outer part of soil	S1	11	S1CDB1-S1CDB11
2	Scrapping the food waste	S2	6	S2CDB1-S2CDB6
3	Different peels of unprocessed food	S3	6	S3CDB1-S3CDB6
4	From different combination of decomposed wastes	S4	7	S4CDB1-S1CDB7

Table 1: Different Sample Sites Selected for Sample Collection to Isolate CDB

3.2 Screening of Cellulose Degrading Bacterial Isolates

Potential cellulose-degrading isolates were screened by applying 1% Congo red dye to colonies grown on CMC agar plates. All 30 bacterial isolates showed clear zones around their colonies, indicating their ability to degrade cellulose. The cellulolytic index (CI) was calculated for each isolate by dividing the diameter of the hydrolyzed zone by the diameter of the colony. Among these, 10 isolates had a zone-to-colony ratio of 1.5 or higher (Tables 2 and 3). The isolates were classified based on their cellulolytic activity as follows: high cellulolytic activity (CI range 3.1-4.0) included S1CDB1 and S4CDB3; moderate cellulolytic activity (CI range 2.1-3.0) included S2CDB2, S3CDB1, and S4CDB1; and lower cellulolytic activity (CI range 1.1-2.0) included S1CDB2, S1CDB3, S2CDB1, S4CDB2, and S4CDB4, according to Bashir [12]. Based on their CI values, four isolates (S1CDB1, S2CDB2, S3CDB1, and S4CDB3) were identified as the most efficient and selected for further identification tests. The highest cellulolytic index was 4.03 (S1CDB1), while the lowest was 2.50 (S2CDB2). Notably, isolates S1CDB1 and S4CDB3 exhibited the highest cellulolytic activities.

Cellulolytic Bacteria	Lower Cellulolytic	Moderate Cellulolytic	High Cellulolytic	Significant Cellulolytic
Ratio > 1.5 10	Activity Ratio 1-2.6	Activity Ratio 2.1-3.2	Activity Ratio 3.1-4.2	Activity Ratio 4.1-5.5 0

Table 2: Activity Ranges of Cellulolytic Bacterial Isolates (ZD/CD Ratio) Used for Classification

Name of isolates	Cellulolytic Index (CI)
S1CDB1	$4.03 + 0.06^{h}$
S1CDB2	$1.50 + 0.01^{a}$
S1CDB3	$2.00 + 0.01^{c}$
S2CDB1	$2.00 + 0.01^{c}$
S2CDB2	$2.50 + 0.10^{e}$
S3CDB1	$2.60 + 0.10^{f}$
S4CDB1	$1.20 + 0.01^{a}$
S4CDB3	$3.50 + 0.10^{g}$
S4CDB2	$1.75 + 0.01^{b}$
S4CDB4	$1.54 + 0.01^{a}$

Table 3: Cellulolytic Index Values for the CDB Isolates

"Superscripts above CI values indicate significant differences at the $p < 0.05$ level."

3.3 Characterization of Cellulolytic Bacteria

3.3.1 Morphological Characterization

The morphological characteristics of the isolates were determined by examining the physical features of their colonies (Table 4). The bacterial isolates S1CDB1 and S3CDB1 had large colonies, while S2CDB2 and S4CDB3 had medium-sized colonies. All colonies were circular, with three isolates (S1CDB1, S2CDB2, and S3CDB1) appearing white, and one isolate (S4CDB3) appearing whitish-yellow. The Gram reaction property of the isolates (S1CDB1, S2CDB2, S3CDB1, and S4CDB3) was assessed using the non-staining (KOH) test, and all isolates were found to be Gram-negative. Additionally, all test isolates exhibited growth along the stabbed line in the media without any turbidity formation, indicating that they are non-motile and lack flagella.

Isolates name	Colony pigment	Shape	Size	KOH test	Motility Test
S1CDB1	White	Circular	Large sized	Gram negative	Non motile
S2CDB2	White	Circular	Medium sized	Gram negative	Non motile
S3CDB1	White	Circular	Large sized	Gram negative	Non motile
S4CDB3	Whitish yellow	Circular	Medium sized	Gram negative	Non motile

Table 4: Morphological Characterization of CDB Isolates

3.3.2 Biochemical Characterization

The selected isolates were biochemically characterized and tested for various reactions including oxidase, catalase, methyl red, citrate utilization, starch hydrolysis, urease, and Voges-Proskauer (Figure 1; Table 5). All isolates tested negative for catalase, as no bubble formation was observed. This result is consistent with findings that catalase is present in most cytochrome-containing aerobic and facultative anaerobic bacteria, but not in Streptococcus species [13]. For the citrate utilization test, the medium changed to blue after incubation, indicating a positive result and showing that all selected isolates were capable of using citrate as their sole carbon source and ammonia as their sole nitrogen source.

Two of the isolates (S2CDB2 and S3CDB1) tested positive for starch hydrolysis, while the other two isolates (S1CDB1 and S4CDB3) tested negative. All cellulose-degrading bacterial isolates were positive for urease, as evidenced by the media changing color from yellow to pink, demonstrating their ability to utilize urea [14]. The study also found that all CDB isolates were oxidase-negative, as indicated by the absence of color change after mixing the colonies with the oxidase reagent on tissue paper. This result is consistent with findings that genera of the Enterobacteriaceae family are typically oxidase-negative [15]. In the absence of the enzyme, the reagent remains reduced and colorless, indicating a negative result [16].

All test isolates showed a color change from bright yellow to red after the addition of methyl red reagent, indicating that all isolates were methyl red positive. In contrast, the isolates were negative for the Voges-Proskauer (VP) test, which assesses the conversion of glucose to acetylmethylcarbinol. If glucose is metabolized, it reacts with alpha-naphthol (VP reagent 1) and potassium hydroxide (VP reagent 2) to produce a red color [15].

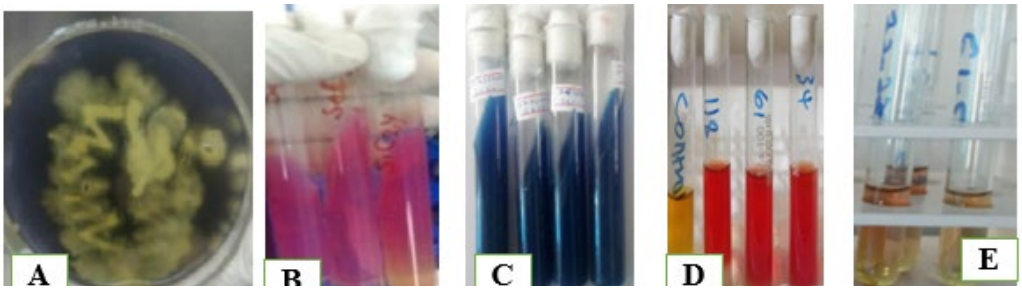

Figure 1: Biochemical Characterization of CDB Isolates: (A); Positive Starch Hydrolysis Test;(B): Positive Urease Test; (C): Positive Citrate Utilization Test; (D): Positive Methyl Red Test, and (E): Negative Vogues-Proskauer's Test

Biochemical tests	CDB isolates			
	S1CDB1	S2CDB2	S3CDB1	S4CDB3
Motility Test	Non-motile	Non motile	Non motile	Non motile
Catalase Test	-	-	-	-
Citrate Utilization Test	+	+	+	+
Starch hydrolysis:	-	++	+++	-
Urease taste:	+	+	+	+
Oxidase test	-	-	-	-
MR test	+	+	+	+
VP test	-	-	-	-
+ =positive; ++ =medium positive; +++ =highly positive; - =negative; MR= methyl red; and VP= Vogues Proskauer.				

Table 5:The Biochemical Tests for CDB Isolates

3.4 Determination of Optimal pH

All four bacterial isolates were tested to determine their optimum pH for growth, using a range from pH 5 to 8 (Figure 2). The results indicated that the optimum pH for the growth of the CDB was 7.0. All isolates exhibited moderate growth between pH 6.0 and 7.0. Specifically, isolate S2CDB2 showed maximum growth at pH 6, while isolate S4CDB3 showed low growth at lower pH ranges but maximum growth at pH 8. Other isolates experienced decreased growth at pH 8. These findings are consistent with the results of Balamurugan, and similar studies have indicated that optimum conditions for cellulase production by Bacillus sp. W12 are observed at pH 7 [17,18].

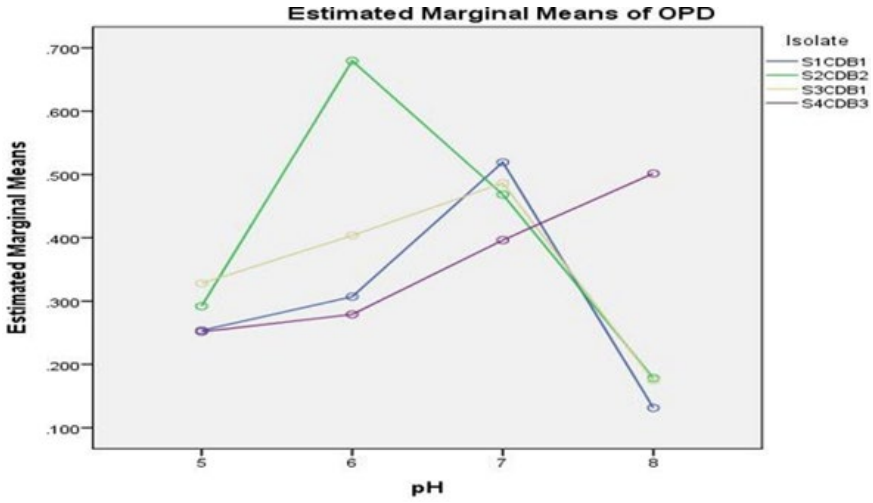

Figure 2: Illustration Graph for Growth Potential of CDB Isolates vs. pH Ranges

3.5 Determination of Optimal Temperature

Four bacterial isolates were examined to determine their optimum temperature for growth across a range from 30°C to 50°C (Figure 3). The results indicated that most isolates grew well between 35°C and 40°C. The maximum growth for the S1CDB1 isolate was observed at 35°C. All isolates showed a decline in growth as the temperature increased beyond this range [17]. Balamurugan *et al.* (2011) noted that CDB isolates growing between 30°C and 40°C are typically mesophilic. However, this study also found that the isolates could grow at 45°C and 50°C, suggesting a thermophilic nature. Microbial cellulases from various sources have been reported to have an optimum temperature of approximately 35-40°C [19,20]. A similar temperature profile was observed in a study involving Bacillus licheniformis [9].

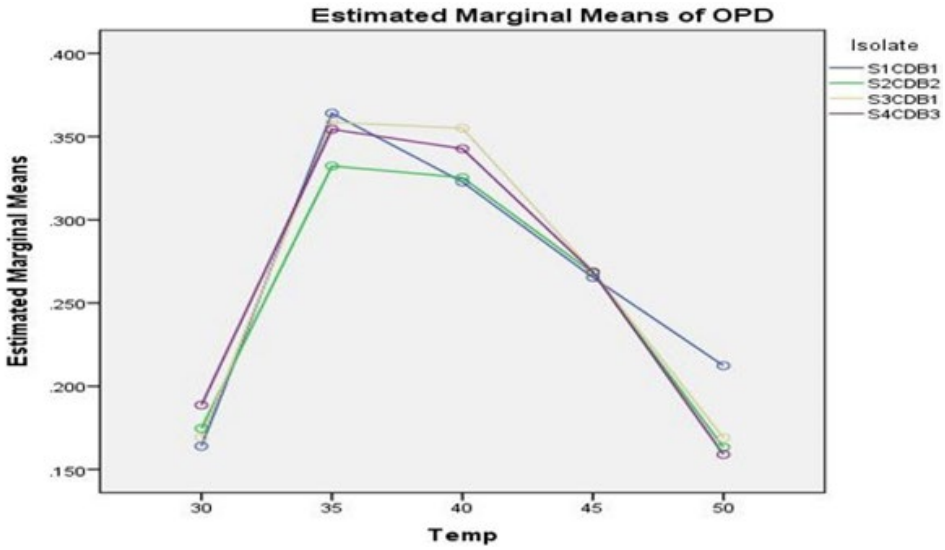

Figure 3: Illustration Graph for Growth Potential of CDB Isolates vs. Temperature Ranges

3.6 Heavy Metal Tolerance Assay

The heavy metal tolerance assay was conducted for four CDB isolates using various concentrations of mercury, ranging from 0.01% to 0.05% (Figure 4). All isolates demonstrated considerable growth at concentrations of 0.01% and 0.02%, with no statistically significant variation at the 0.05 significance level. The isolates could tolerate mercury concentrations up to 0.04%, but their growth declined at 0.05%. reported that S. epidermidis and E. coli exhibited metal tolerance up to 1.5 mg/ml for nickel, 3 mg/ml for zinc, 3 mg/ml for lead, 1.5 mg/ml for cadmium, and 0.9 mg/ml for iron. These bacterial isolates, attributed to the Gram-negative Enterobacteriaceae family, showed similar heavy metal tolerance characteristics [21].

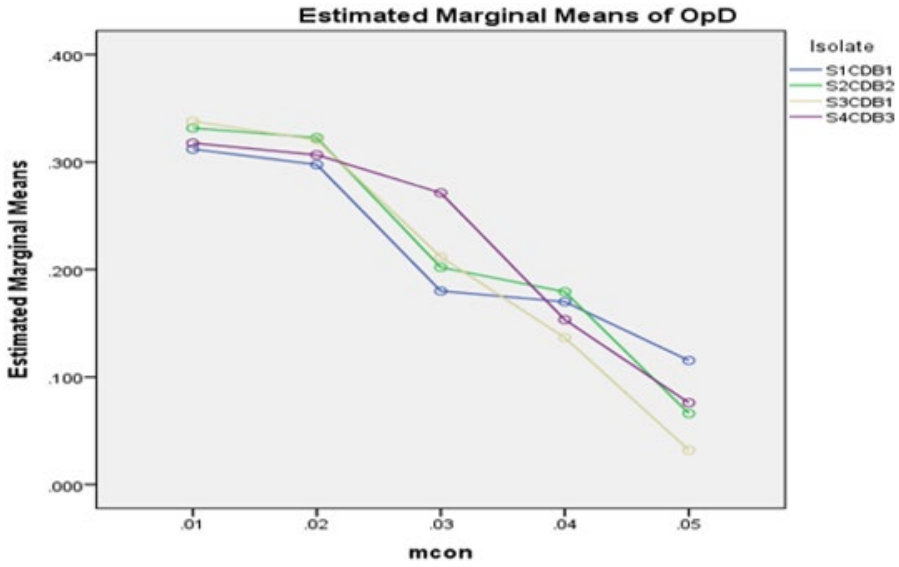

Figure 4: Illustration Graph for Growth Potential of CDB Isolates vs. Heavy Metal (Hg) Concentrations

4 Conclusion

Food waste presents a valuable opportunity for screening cellulolytic bacteria. This study aimed to identify and characterize cellulose-degrading bacteria from food waste collected from a students' cafeteria disposal site. Four effective cellulolytic bacterial isolates were successfully obtained and analyzed for their morphological and biochemical properties. The results highlight the potential of these isolates for use in bioethanol production and other industrial applications. The functional tests revealed that the isolates exhibit both mesophilic and thermophilic temperature characteristics, with optimal growth observed at temperatures ranging from 35°C to 40°C. Additionally, the isolates thrived at a neutral pH of 7 and demonstrated tolerance to mercury concentrations ranging from 0.01% to 0.05%. These findings underscore the promising cellulolytic activities of the CDB isolates. Further research is needed to conduct molecular characterization and assess cellulase enzyme production to accurately identify these isolates at the species level and fully understand their cellulolytic capabilities.

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