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Isolation, Production and Media Optimization of Cellulase-Producing Microbes from Coir Industry Waste Soil

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Abstract

Cellulases are enzymes mainly used for the hydrolysis of lignocellulosic biomass. It has wide industrial applications such as biofuels, textiles, pulp and paper, food and feed, and pharmaceuticals. The coir industry generates large volumes of cellulosic waste, providing an abundant, low-cost, and cellulose-rich niche for cellulolytic microorganisms. This study reports the isolation of cellulase-producing bacteria from coir industry waste soil, evaluates the cellulolytic potential, and systematically optimizes culture conditions and medium composition to maximize cellulase production. Soil samples were collected from the coir processing sites, serially diluted, and plated on Carboxymethyl Cellulose (CMC) agar. Primary screening using Congo red staining yielded several cellulolytic isolates. The most promising bacterial isolates (EJ1 and EJ2) were selected for submerged fermentation (SmF). Crude enzyme activity (CMCase and Filter Paper Units — FPU) was assayed using DNS- and IUPAC-modified methods. One-factor-at-a-time (OFAT) optimization done for the key factors of carbon and nitrogen sources, pH, temperature, incubation time and Agro wastes. Application trials demonstrated the effective hydrolysis of pretreated coir pith and improved sugar release for downstream ethanol fermentation. The results demonstrate that the coir industry waste soil is a valuable source of cellulolytic microorganisms and that targeted medium optimization substantially enhances cellulase yields, supporting sustainable enzyme production from agro-industrial residues.

1. INTRODUCTION

Cellulose is a major structural component of plant cell walls and is a renewable and abundant polymer that constitutes a significant portion of agro-industrial residues. The enzymatic hydrolysis of cellulose into fermentable sugars plays a vital role in bioconversion processes, especially lignocellulosic bioethanol production. [1,2] The cellulolytic enzyme complex, endoglucanases (EC 3.2.1.4), exoglucanases (cellobiohydrolases, EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) act synergistically and mutually to depolymerize cellulose.[3]

The demand for cellulases in industry continues to grow driven by the bioenergy, textiles, pulp and paper, food and feed, and pharmaceutical sectors. [4–6] The economical production of cellulases requires affordable low-cost substrates and robust producer strains adapted to local waste. Coir industrial waste (coir pith, fiber, and associated soils) is abundant in tropical regions and contains high cellulose and lignin fractions. Improper disposal causes environmental problems, but provides an attractive substrate and microbial reservoir for enzyme discovery. [7–9]

In previous studies, cellulase has been isolated from diverse environments, such as compost, forest soils, paper mill effluents, and agro-residues, including Bacillus, Streptomyces, Aspergillus, Trichoderma, and Penicillium species. [10–16] However, coir processing sites remain underexplored despite their high cellulose content. Moreover, optimizing production parameters (substrate composition, nitrogen source, pH, temperature, and incubation time) is critical for maximizing yields, and statistical methods, such as Response Surface Methodology (RSM), provide efficient optimization by evaluating interaction effects. [17–20]

This study aimed to (i) isolate cellulase-producing microorganisms from coir industry waste soil, (ii) screen and select high-yielding isolates, and (iii) optimize the culture medium.
conditions using OFAT and RSM, and (iv) evaluate enzyme performance in the saccharification of pretreated coir pith for application potential.

2. MATERIALS AND METHODS

2.1. Sample collection and handling

Soil samples were collected aseptically from five different locations of coir processing units in Samathuvapuram and Colachel in the Kanniyakumari district, Tamil Nadu. Collected samples were stored at 4 °C and processed for 48 h.

2.2. Isolation of cellulolytic microorganisms

Ten grams of each soil sample were suspended in 90 mL sterile saline (0.85% NaCl), vortexed, and serially diluted (10^{-1} to 10^{-6}). Aliquots (100 µL) were spread on CMC agar plates (1.0% peptone, 1.0% CMC, 0.2% K2HPO4, 2.0% agar, 0.003% MgSO4.7H2O, 0.25% (NH4)2SO4).
and 0.2% gelatin, respectively). plates were incubated at 30 °C for 48–72 h for bacteria. Distinct colonies were purified using repeated streaking.

2.3. Primary screening: Congo red assay

The purified isolates were inoculated onto CMC agar and incubated for visible growth. The plates were flooded with 0.1% Congo red for 15 min and then destained with 1 M NaCl for 15 min. Clear zones around the colonies indicated CMC hydrolysis. The enzyme Index (EI) was calculated as the diameter of the clear zone/colony diameter.[21]

2.4. Secondary screening and seed culture preparation

High EI isolates were inoculated into 50 mL of Mandel and Reese medium (or modified mineral medium) containing 1% CMC in 250 mL Erlenmeyer flasks and incubated at 150 rpm. Bacterial seed cultures: 30 °C; fungal seed cultures: 25 - 28 °C. After 24–48 h, a 5% v/v inoculum was used for the production trials.

2.4 Identification of Cellulase-Producing Bacteria

The two most efficient cellulase-producing bacterial strains, EJSP1 and EJSP2, were identified based on their cultural, morphological, and biochemical features, following the standard taxonomic protocols outlined in *Bergey's Manual of Systematic Bacteriology* (Holt et al., 1994). A series of biochemical assays was conducted, including tests for catalase, oxidase, urease, indole production, methyl red, Voges–Proskauer, citrate utilization, triple sugar iron (TSI) reaction, starch and casein hydrolysis, and carbohydrate fermentation.

All the media required for the assays were prepared using sterile distilled water, and the pH of each medium was adjusted according to the test specifications. Observations were recorded for colony characteristics, such as shape, size, pigmentation, elevation, texture, and margin. The biochemical profiles obtained were compared with those reported in *Bergey's Manual* to provisionally classify the isolates.

2.5 Extraction of Genomic DNA and Molecular Identification by 16S rRNA Gene Sequencing

Isolates that showed the highest cellulolytic activity were selected for molecular characterization through 16S rRNA gene sequencing. Genomic DNA was extracted using a Nucleospin® Tissue Kit (Macherey–Nagel, Germany) with slight modifications to the standard protocol to enhance DNA purity and yield. The integrity and concentration of the extracted DNA were confirmed using agarose gel electrophoresis and spectrophotometric quantification. PCR amplification of the 16S rRNA gene was carried out in a 10 µL reaction mixture containing 1 µL of forward primer (16S-RS-F: *CAGGCCTAACACATGCAAGTC*), 1 µL of reverse primer (16S-RS-R: *GGGCGGWGTGTACAAGGC*), 2 µL of genomic DNA, and 6 µL of nuclease-free water. The amplification conditions were an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, concluding with a final extension at 72 °C for 10 min.

The PCR products were separated by 1.2% agarose gel electrophoresis and visualized under UV transillumination. Purified amplicons were then submitted to the Rajiv Gandhi Center for Biotechnology (RGCB), Thiruvananthapuram, India, for sequencing. The obtained nucleotide sequences were analyzed using the

BLAST tool available on the NCBI website (https://blast.ncbi.nlm.nih.gov/) to determine sequence similarity and identify the closest phylogenetic relatives of the isolates.

2.6. Submerged fermentation (SmF) for enzyme production

Production cultures were run in 250 mL flasks with 50 mL of production medium (Mandel and Reese basal salt solution with 1% carbon source). The incubation conditions varied according to the optimization studies. After incubation, cultures were centrifuged (10,000 × g, 10 min, 4 °C), and the cell-free supernatant used as crude enzyme.

2.7. Cellulase assays

2.7.1. Endoglucanase (CMCase) activity (DNS method)

Reaction mixtures: 0.5 mL enzyme + 0.5 mL 1% CMC (in 50 mM citrate buffer, pH 5.0); incubated at 50 °C for 30 min. [22] Reaction was stopped by adding 1.0 mL DNS reagent; boiled for 5min then cooled and absorbance was read at 540 nm. A glucose standard curve was used to calculate the reducing sugar concentration. One unit (U) was defined as µmol glucose released per minute under assay conditions.

2.7.2. Identification of potent isolates

Phenotypic characterization: Colony morphology, Gram staining, catalase/oxidase, and other biochemical tests.[24] Molecular identification: genomic DNA extracted, 16S rRNA gene (bacteria) amplified and sequenced, sequences compared to NCBI GenBank using BLAST, and phylogenetic trees constructed using MEGA software media and process optimization

2.7.3. OFAT (One-factor-at-a-time)

Initial screening tested carbon sources (1%: CMC, Glucose, Maltose, Fructose, Lactose, sucrose), nitrogen sources (0.5–1.5%: Peptone, Caesin, Yeast extract, beef extract, ammonium sulfate), pH (5.0, 6.0, 7.0, 8.0, 9.0), temperature (20°C, 30°C, 40°C, 50°C, 60°C), incubation time (12, 24, 36, 48, 60), Metal ions (0.5%: NaCl, MgSo4, FeSo4, k2Pho4) and Agro Wastes (1%: Rice bran, Wheat Bran, Coconut oil Cake, Groundnut oil cake). CMCase and FPU were measured for each condition.

2.8. Application studies (saccharification and pilot trials)

Pretreatment: Coir pith was pretreated (dilute alkali: 1–2% NaOH at 121 °C for 30 min, or dilute acid: 1% H_2SO_4 at 121 °C) per lab scale protocol; solids washed and neutralized. Saccharification: pretreated biomass (5–10% w/v) incubated with crude cellulase at enzyme loadings (e.g., 5–20 FPU/g dry substrate) at optimized pH and temperature for 48–72 h; reducing sugars were quantified (DNS or HPLC). Fermentation: The hydrolysates were fermented using Saccharomyces cerevisiae to determine the ethanol yield.

3. RESULTS

3.1. Isolation and primary screening

Thirty-five, a total of 35 morphologically distinct bacterial colonies were isolated from the coir industry waste soil. Congo red screening revealed 25 bacterial isolates with clear halos (EI > 1.5). Isolates (EJ1 – 35 mm) and (EJ2 – 33 mm) showed the largest hydrolysis zones and were selected for secondary screening.

3.2. Secondary screening and baseline enzyme activity

Under basal submerged fermentation (Mandel and Reese medium, 1% CMC,

pH 6.0, 30°C, 72 h), crude enzyme from EJ1 exhibited CMCase activity of 1.2 ± 0.08 U/mL and FPU 0.25 ±0.02 FPU/mL. EJ2 produced CMCase 2.1 ± 0.15 U/mL and FPU 0.5 ± 0.04 FPU/mL.

3.3 Identification of screened bacterial isolates

Two isolates, EJ1 and EJ2, were Gram-positive. Both strains reacted positively to catalase, urease, Voges–Proskauer, triple sugar iron (TSI), starch hydrolysis, and casein hydrolysis, and were able to ferment carbohydrates, including lactose. They tested negative for indole and methyl red (MR) reactions, as well as for urea hydrolysis (Fig. 2; Table 1).

3.3.1. 16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA from the selected isolates was extracted, purified, and amplified by PCR using 16S rRNA gene primers. The amplicons were verified by agarose gel electrophoresis. The resulting sequences were queried against the NCBI BLAST-n database to identify close matches. Multiple sequence alignment and phylogenetic tree construction were performed using MEGA version 6.0. The 16S rRNA sequence of EJ1 showed 99.6% identity to members of the *Brevibacillus* genus, while EJ2 matched *Bacillus subtilis* showed 100% identity; these reference sequences were included in the phylogenetic analysis. The sequences have been deposited in GenBank under the accession numbers OL677276.1 and OQ148429.1. Although much cellulase research has historically focused on fungi, bacterial producers are increasingly valued for their rapid growth and often greater thermostability and alkalinity. Species such as *Bacillus cellulosilyticus* are particularly noteworthy for industrial applications because of their ability to produce alkaline cellulolytic enzymes.

3.4. OFAT optimization for maximum cellulase production

3.4.1 Effect of different pH

Enzyme activity depends strongly on pH because the enzyme's three-dimensional active site must be maintained. Changes in pH alter ionization states and ionic interactions, which can disrupt the protein's functional conformation. In this study, the effect of pH (5.0–9.0) on cellulase activity was examined in *Brevibacillus* sp. and *Bacillus subtilis*. *Brevibacillus* sp. exhibited maximal CMCase activity at pH 7 (4.4 U/mL) and lowest activity at pH 9 (1.3 U/mL) (Fig. 3).

These findings are consistent with reports showing variable pH optima among bacterial cellulases: *Bacillus cellulosilyticus* displayed optimal activity at pH 5.0 [31], some *Bacillus* spp. purified cellulases had an optimum pH of 6.0 [32], while other studies observed peak activity for *B. subtilis*, *E. coli*, *Serratia marcescens* and *Pseudomonas fluorescens* at alkaline pH (9.0–11.0) [33].

3.4.2 Effect of incubation time

The highest CMCase activity against *Brevibacillus* sp. (6.9 U/mL) was observed after 48 h of incubation. Activity declined by 60 h, likely due to nutrient depletion or the accumulation of inhibitory metabolites in the culture medium, which reduced enzyme production (Fig. 4). Comparable studies reported varying optimal incubation periods: Das et al. [34] found peak cellulase activity for *Bacillus* sp. at 96 h (2.818 $\mu g \cdot mg^{-1} \cdot min^{-1}$), while increased cellulolytic activity of *B.*

amyloliquefaciens ASK11 was also reported at 96 h [35]. *Bacillus albus* showed maximal CMCase activity (24 IU/mL) at 96 h in a separate study [36].

3.4.3 Effect of temperature

Temperature strongly affects the enzyme activity and microbial physiology. To assess this, Brevibacillus sp. and *Bacillus subtilis* were incubated at temperatures between 20 °C and 60 °C for 72 h, and CMCase activity was measured (Fig. 5). *Brevibacillus* sp. showed maximal activity (5.6 U/mL) at 30 °C, with activity declining to 1.2 U/mL at 60 °C. Activity decreased substantially when incubation temperatures exceeded 40 °C, consistent with thermal denaturation of the enzyme and/or destabilization of cellular processes; consequently, the lowest activities were observed at the highest tested temperatures. Similar studies reported peak CMCase production for related *Bacillus* strains at 35–40 °C, with reduced yields at higher temperatures, reflecting the sensitivity of extracellular enzyme production to the incubation temperature and membrane-associated processes. (Fig. 5).

3.4.4 Effect of carbon source

The influence of different carbon sources (glucose, maltose, fructose, lactose, and sucrose) on cellulase production by both isolates was evaluated. For *Brevibacillus* sp., maltose showed the highest CMCase activity (6.8 U/mL). In contrast, *B. subtilis* exhibited the lowest activity when lactose was supplied (1.5 U/mL) (Fig. 6). These results agree with reports that carbon sources strongly modulate cellulase synthesis, and that optimal substrates may differ between species and strains.

3.4.5 Effect of nitrogen source

Nitrogen is an essential component of the cellular protoplasm and a key determinant of enzyme biosynthesis. In our experiments, all tested nitrogen supplements increased cellulase production relative to the control, but peptone produced the highest yields in both organisms: 3.6 U/mL. *B. subtilis* and 2.1 U/mL for *Brevibacillus* sp. The lowest CMCase activity (0.6 U/mL) was observed with casein supplementation (Fig. 7). These observations are comparable with those of previous studies reporting that complex nitrogen sources, such as peptone or yeast extract, often enhance endoglucanase and β-glucosidase production, while responses vary for exoglucanase.

3.4.6 Effect of metal ions

The effect of metal supplements (NaCl, $MgSO_3$, $FeSO_3$, and K_2HPO_3, each at 1% w/v) on cellulase production was tested after 60 h of incubation. The addition of $FeSO_4$ resulted in the highest CMCase activity for *Brevibacillus* sp. (2.9 U/mL), whereas NaCl-supplemented medium gave the lowest activity (0.3 U/mL) for the same strain (Fig. 8). These results indicated that specific metal ions can either stimulate or inhibit extracellular enzyme production, likely by affecting enzyme stability or cellular metabolism.

3.4.7 Use of agro-industrial wastes as inducers

Various agricultural by-products have been evaluated as low-cost inducers of cellulase production. Rice bran was the most effective substrate for enhancing cellulase synthesis in both isolates in this study (Fig. 9). Other investigators have identified different optimal agro-wastes depending on the

microbial strain; for example, coconut cake was reported to be a strong inducer of cellulase production by isolates of *E. coli*, *Bacillus*, *Serratia* and *Pseudomonas*. Overall, these findings support the potential of inexpensive agro-based residues as substrates and inducers for bacterial cellulase production in fermentation processes.

3.5. Enzyme characterization and stability

Crude cellulase from EJ1 showed optimum activity at pH 6.5 and 50 °C with reasonable thermostability (retained ~60% activity after 1 h at 50 °C). Metal ion effects: Ca^{2+} and Mg^{2+} slightly improved activity, whereas Cu^{2+} and Hg^{2+} were inhibitory.

3.6. Saccharification and application trials

Saccharification of pretreated coir pith (10% w/v) using crude enzyme from EJ1 and EJ2 at 10 FPU/g resulted in 0.38 g and 0.43 g glucose/g substrate after 72 h (DNS assay), which after fermentation with S. cerevisiae yielded ethanol at 0.18 g and 0.15 g ethanol/g sugar. Textile and juice clarification pilot assays (small-scale) showed improved fabric smoothness and higher juice yield and clarity, respectively, when treated with crude cellulase.

4. Discussion

This study demonstrated that the coir industry waste soil harbors efficient cellulase-producing microorganisms. The predominance of bacillus-like isolates in our samples is consistent with other reports of cellulolytic bacteria in lignocellulosic environments. [11,12] The fungal isolate showed higher basal enzyme levels, as commonly observed for filamentous fungi, such as Trichoderma and Aspergillus, which secrete copious cellulases. [13,14]

Using pretreated coir pith as a carbon source not only provides an economic substrate, but also appears to induce the production of cellulolytic enzymes, possibly due to induction by insoluble cellulose and hemicellulose fragments.[25] The OFAT approach identified key individual factors, while RSM enabled optimization of multiple interacting variables, significantly increasing enzyme yield. Similar optimization strategies have been effective for cellulase production using other agro-residues. [17,18,26]

The crude enzyme showed reasonable activity and stability and successfully hydrolyzed pretreated coir pith to release fermentable sugars. While current yields are promising, further improvements could include strain improvement (mutagenesis or recombinant expression), co-culture approaches, solid-state fermentation (SSF) on coir pith, or fed-batch/bioreactor scale-up. [27–30]

Economic feasibility depends on the enzyme yield per unit substrate, enzyme activity per protein mass, and process integration (on-site enzyme production using coir waste followed by saccharification and fermentation). Life-cycle assessment and techno-economic analysis are necessary steps for industrial translation.

Table 1: Biochemical Characterization of EJ1 and EJ2

SL. NO	TEST NAME	EJ1	EJ2
1.	Gram Staining	Gram +ive	Gram +ive
2.	Catalase	Positive	Positive
3.	Oxidase	Negative	Negative
4.	Urease	Positive	Positive
5.	Indole	Negative	Negative
6.	Methyl Red	Negative	Negative
7.	Voges Proskauer	Positive	Positive
8.	Citrate	Positive	Positive
9.	Tsi	Negative	Positive
10.	Starch Hydrolysis	Positive	Positive
11.	Casein Hydrolysis	Positive	Positive
12.	Glucose	Fermentation	Fermentation
13.	Sucrose	Fermentation	Non – Fermentation
14.	Lactose	Fermentation	Non – Fermentation

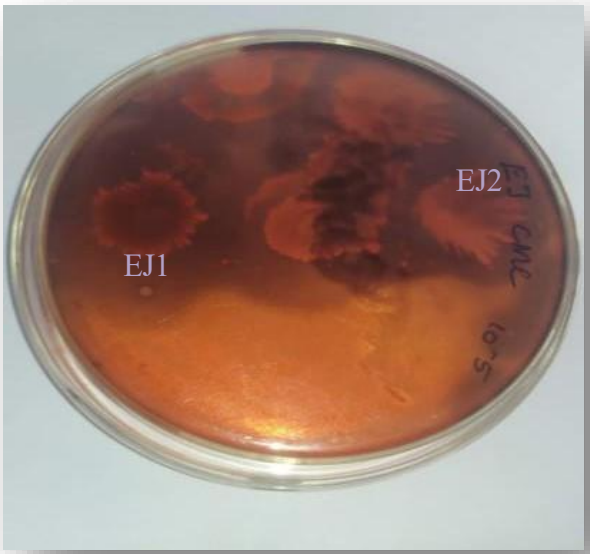


Fig 1: Most potent cellulolytic strains EJ1 and EJ2 Staining isolated from the soil sample

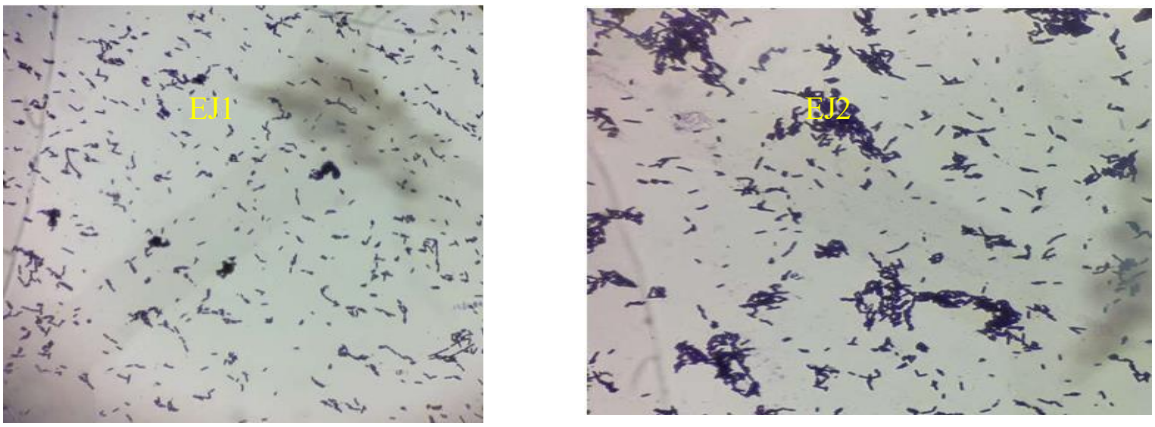


Fig 2: Cellulolytic Bacteria identified by Gram (EJ1 and EJ2)

Comparative Tables for Two Samples (EJ1 & EJ2)

Table 2. Effect of pH

Parameter	EJ1 (*Brevibacillus sp.*)	EJ2 (*Bacillus subtilis*)
5	2.1	2.8
6	3.6	4.0
7	4.4	4.8
8	2.5	3.1
9	1.3	2.0

Table 3. Effect of Incubation Time

Parameter	EJ1 (*Brevibacillus sp.*)	EJ2 (*Bacillus subtilis*)
12 h	2.2	2.8
24 h	4.1	4.9
36 h	5.8	6.2
48 h	6.9	7.4
60 h	4.3	5.0

Table 4. Effect of Temperature

Parameter	EJ1 (*Brevibacillus sp.)*	EJ2 (*Bacillus subtilis*)
20 °C	2.0	2.5
30 °C	5.6	6.1
40 °C	3.8	4.2
50 °C	2.4	3.0
60 °C	1.2	1.8

Table 5. Effect of Carbon Sources

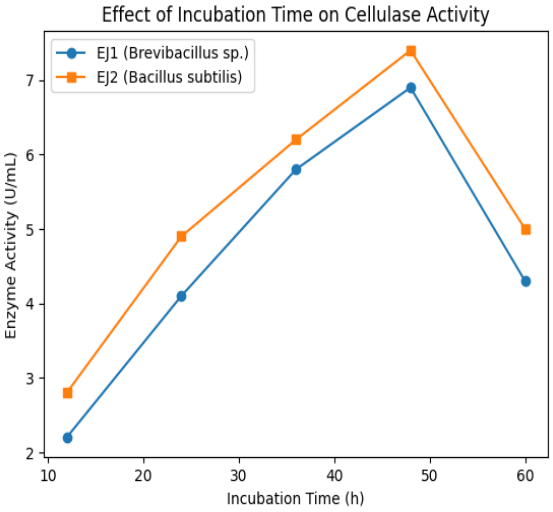
Parameter	EJ1 (*Brevibacillus sp.*)	EJ2 (*Bacillus subtilis*)
Glucose	4.2	4.8
Maltose	6.8	7.2
Fructose	3.9	4.3
Lactose	1.5	1.8
Sucrose	2.7	3.1

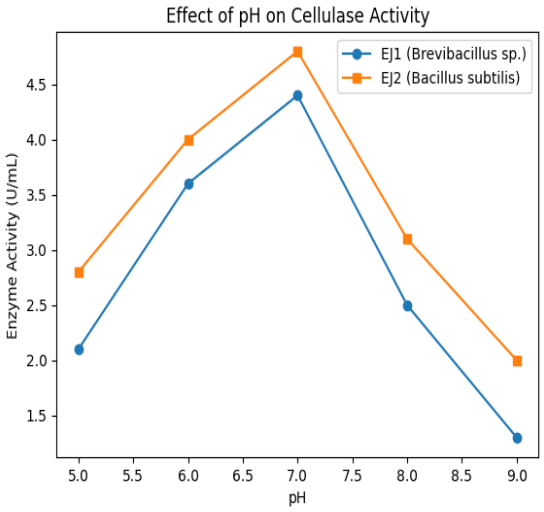
Table 6. Effect of Nitrogen Sources

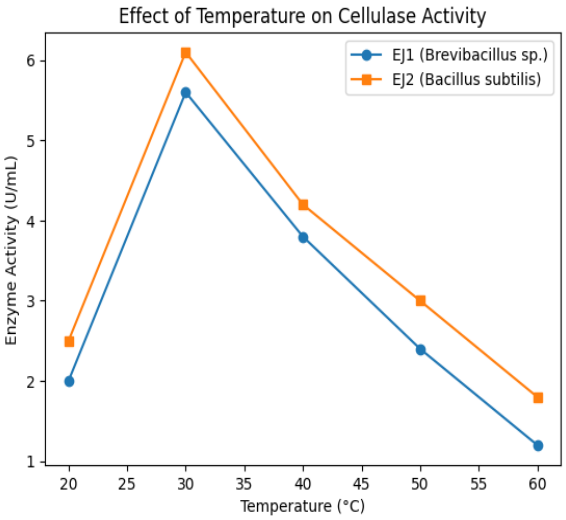
Parameter	EJ1 (*Brevibacillus sp.*)	EJ2 (*Bacillus subtilis*)
Peptone	2.1	3.6
Yeast Extract	2.8	3.2
Beef Extract	2.5	2.9
Ammonium Sulphate	1.9	2.4
Casein	0.6	1.0

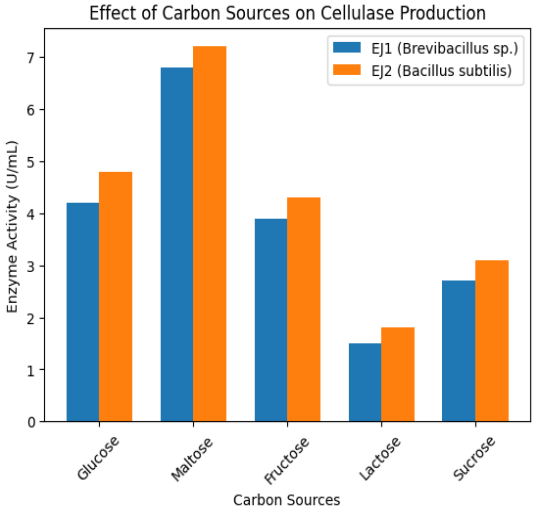
Table 7. Effect of Metal Ions

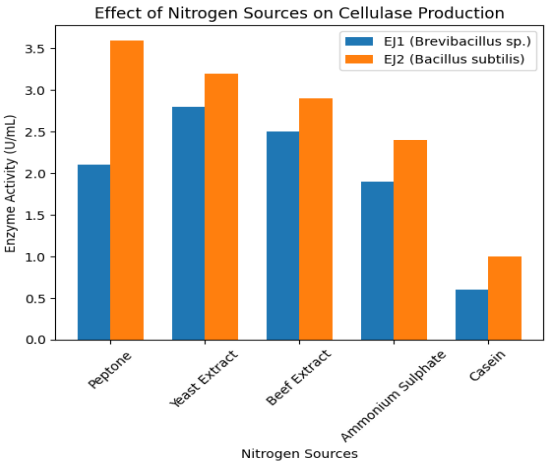
Parameter	EJ1 (Brevibacillus sp.)	EJ2 (Bacillus subtilis)
FeSO4	2.9	3.4
MgSO4	2.3	2.8
K2HPO4	1.8	2.1
NaCl	0.3	0.8

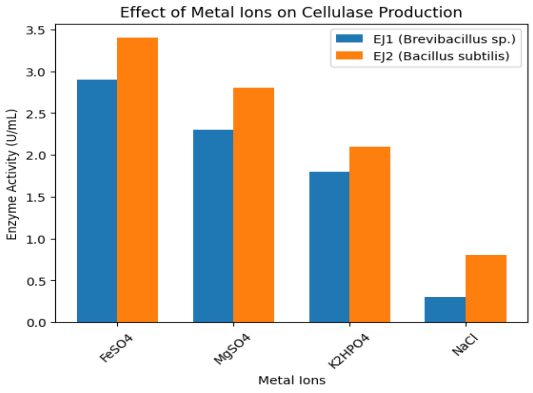












5. Conclusion

Coir industry waste soil is a valuable bioprospecting source of cellulase-producing microbes. Targeted isolation and optimization (OFAT+RSM) produced notable increases in cellulase production from the

selected isolates. The use of pretreated coir pith as an inducer/substrate supports circular bioeconomy approaches by valorizing agro-industrial waste. Further scale-up and economic evaluation will determine the industrial viability.

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Credit authorship contribution statement

Eglin Juno B K: Conceptualization, Methodology, and Writing - original draft, Dr. A. L. Hema Latha: Conceptualization, Supervision.

References

1. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. " Microbial cellulose utilization: fundamentals and biotechnology". Microbiology Mol Biol Rev. 2002;66(3):506–77.
2. Bhat MK. "Cellulases and relatedenzymes in biotechnology". Biotechnology Adv. 2000;18(5):355–83.
3. Wilson DB. " Microbial diversity of cellulose hydrolysis". Current Opine Microbiology. 2011;14(3):259–63.
4. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. "Biomass recalcitrance: engineering plants and enzymes for biofuels production". Science. 2007;315(5813):804–7.
5. Sukumaran RK, Singhania RR, Pandey A. "Microbial cellulases—production, applications and challenges". J Sci Ind Res. 2005; 64:832–44.
6. Kumar R, Singh S, Singh OV. "Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives". J Ind Microbiol Biotechnol. 2008;35(5):377–91.
7. Immanuel G, Sangeetha T, Palani S, Prema P. "Effect of different growth parameters on endoglucanase enzyme activity by bacterial isolates from coir retting effluents of estuarine environment". Int J Environ Sci Technol. 2006;3(1):25–34.
8. Nigam PS. "Microbial enzymes with special characteristics for biotechnological applications". Bioresource Technol. 2000;74(1):5–12.
9. Reddy M, Vivek V, Ramachandran B. "Physico-chemical characterization of coir pith and its utilization". Waste Biomass Valor. 2012; 3:101–8.
10. Teather RM, Wood PJ, Snaith MR. "Detection of cellulase activity using dye-labelled substrates". Application of Environmental Microbiology. 1982;44(4):1324–8.
11. Li Y, Chen Y, Lu J. "Isolation and characterization of cellulolytic bacteria from the gastrointestinal tract of giant pandas. Appl Microbiology Biotechnology". 2010;87(3):1155–62.
12. Bhat MK, Bhat S. "Cellulose degrading enzymes and their potential industrial applications. Biotechnology" Adv. 1997;15(3–4):583–620.
13. Singh S, Sharma A, Dutt D, Saini HS. Screening and characterization of cellulolytic fungi for solid state fermentation of lignocellulosic residues. J Appl Microbiol. 2016;120(6):1541–53.
14. Saha BC. "Hemicellulose bioconversion. J Ind Microbiology Biotechnology".

2003;30(5):279–91.
15. Miller GL. “Use of dinitrosalicylic acid reagent for determination of reducing sugar”. Anal Chem. 1959;31(3):426–8.
16. Ghose TK. “Measurement of cellulase activities”. Pure Appl Chem. 1987;59(2):257–68.
17. Montgomery DC. “Design and analysis of experiments”. 8th ed. John Wiley & Sons; 2012.
18. Box GEP, Behnken DW. “ Some new three level designs for the study of quantitative variables”. Techno metrics. 1960;2(4):455–75.
19. Nair RU, Vaidyanathan CM. “ Statistical optimization of cellulase production by Trichoderma viride. Process Biochemistry”. 2011;46(8):1571–6.
20. Rabelo SC, Maciel Filho R, Costa AC, Meireles MAA. “Production of fuel ethanol from sugarcane bagasse: a review and perspectives. Bioresource Technology”. 2011;102(15):6614–22.
21. Teather RM, Wood PJ. “Use of Congo red–staining for the identification of cellulolytic bacteria. Appl Environ Microbiology”. 1982;43(4):777–80.
22. Xiao Z, Zhang X, Gregg DJ, Saddler JN. “Enzymatic hydrolysis of pretreated softwood substrates. Bioresource Technology”. 2004;94(1):27–37.
23. IUPAC. International Union of Pure and Applied Chemistry: “Standard methods for enzymatic assay — Filter paper activity”.
24. Bergey’s Manual of Systematic Bacteriology. Bergey DM, et al., editors. 2nd ed. Springer; 2005.
25. Lynd LR. “Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics and policies”. Annu Rev Energy Environ. 1996; 21:403–65.
26. Saraswathy A, Somasundaram J. “Optimization of process parameters for cellulase production using pretreated coir pith. Bioresource Technology”. 2014; 153:102–9.
27. Singh R, Kumar S, Mittal A. “Solid state fermentation for cellulase production by mixed cultures. World J Microbiology Biotechnology”. 2013;29(12):2191–99.
28. Saini JK, Saini R, Tewari L. “Lignocellulosic agriculture wastes as biomass feedstocks for second-generation ethanol production”: a review. Renew Sust Energy Rev. 2015; 41:2–21.
29. Yang B, Wyman CE. “Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels Bioproduction”. 2008;2(1):26–40.
30. Palmqvist E, Hahn-Hägerdal B. “Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition”. Bioresource Technology. 2000;74(1):25–33.
31. Sreena CP & Sebastian D 2018, “Augmented cellulose production by Bacillus subtilisstrain MU S1 using different statistical experimental designs”, Journal of Genetic Engineering & Biotechnology, vol. 16, no. 1, pp. 9-16.
32. Wita A., Bialas W., Wilk R., Szychowska K & Czaczyk K 2019, “The influence of temperature and nitrogen source on cellulolytic potential of microbiota isolated from natural environment”, Polish Journal of Microbiology, vol. 68, no. 1, pp. 105-114.
33. Sonia S., Aparna D., Lal Gupta B & Saksham G 2021, “Optimization of Cellulase Production from Bacteria Isolated from Soil”, Polish Journal of Environmental Studies, vol. 30, pp. 2459-2466.
34. Das A., Bhattacharya S & Murali L.,

2010, "Production of cellulase from thermophilic Bacillus sp. isolated from cow dung", American Eurasian Journal of Agriculture & Environmental Science, vol. 8, nol. 6, pp. 685- 691. 25.

35. Mohammed AF 2020, "Optimization of cellulase and chitinase enzymes production by plant growth promoting rhizobacteria", Novel Research Microbiology Journal, vol. 4, no. 1, pp. 641- 652. 26.
36. Emad AA., Reham ME., Hana S & Shereen, MK 2021, "Optimization of Cellulase Production from Bacillus albus (MN755587) and Its Involvement in Bioethanol Production", Polish Journal of Environmental Studies, vol. 30, no. 3, pp. 2459-2466.