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Isolation And Screening of Cellulolytic Bacteria From Cow Dung for Enhanced Cellulase Production

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Abstract-- **The present study reports the isolation, selective screening, and biochemical characterization of cellulase-producing bacteria from cow dung, an abundant and economically accessible lignocellulosic waste substrate. Fresh cow dung samples were collected aseptically, serially diluted, and cultivated aerobically on nutrient agar. Primary screening on Carboxymethyl Cellulose (CMC) agar, followed by Congo red dye displacement, confirmed extracellular endoglucanase production through the formation of distinct hydrolysis halos around active colonies. Gram staining identified the dominant cellulolytic isolates as Gram-positive, rod-shaped bacteria. Among twenty isolates evaluated, SRCMBK28 (1.2 cm), SRCMBK110 (1.1 cm), SRCMBK23 (1.1 cm), SRCMBK11 (1.0 cm), and SRCMBK22 (1.0 cm) demonstrated the largest hydrolysis zones and were selected for further characterization. Secondary screening in liquid mineral salt medium quantified endoglucanase activity using the 3,5-dinitrosalicylic acid (DNS) colorimetric method. The resulting enzyme production profile followed a typical growth-associated pattern, with peak cellulase activity recorded during the late exponential to early stationary phase. Comprehensive biochemical characterization — encompassing oxidase, catalase, indole, methyl red, Voges–Proskauer, citrate utilization, triple sugar iron, sugar fermentation, urease, and hydrogen sulfide production assays — confirmed the broad metabolic versatility of the selected isolates. These findings establish cow dung as a rich and economically viable source of robust cellulolytic bacteria with significant potential for biofuel production, industrial enzyme applications, and sustainable waste valorization.**

Keywords-- **Cellulase; cow dung; Carboxymethyl Cellulose (CMC); cellulolytic bacteria; Congo red assay; DNS method; biochemical characterization; lignocellulosic biomass; bioethanol; Bacillus**

I. INTRODUCTION

The accelerating global demand for sustainable energy and environmentally responsible industrial processes has intensified research interest in lignocellulosic biomass as a renewable carbon feedstock. Among its principal structural components, cellulose is the most abundant natural biopolymer on Earth, constituting the primary load-bearing framework of plant cell walls.

It is a linear homopolysaccharide composed of β-(1→4)-linked D-glucose units arranged in a highly ordered, crystalline structure, rendering it exceptionally resistant to both enzymatic hydrolysis and chemical depolymerization (Bhardwaj et al., 2021). Despite its near-unlimited global availability in agricultural residues and forestry by-products, efficient bioconversion of cellulose into fermentable sugars remains a major scientific and economic challenge. The complete depolymerization of cellulose requires the synergistic action of a tripartite cellulase system. Endoglucanases (EC 3.2.1.4) randomly cleave internal β-1,4 glycosidic bonds within amorphous regions of the cellulose chain, generating new chain ends. Cellobiohydrolases, or exoglucanases (EC 3.2.1.91), act processively from these termini to liberate the disaccharide cellobiose. β-Glucosidases (EC 3.2.1.21) subsequently convert cellobiose and short-chain cello-oligosaccharides into fermentable glucose, simultaneously relieving end-product inhibition of the upstream enzymes (Ezea, 2025). The coordinated output of this tripartite system enables the saccharification of pretreated biomass into substrates suitable for bioethanol and other bioproduct synthesis (Ali et al., 2026).

Microorganisms are the principal biological agents of natural cellulose recycling. Bacterial genera including *Bacillus*, *Clostridium*, *Pseudomonas*, *Aspergillus*, and *Trichoderma* are widely reported as prolific cellulase producers with demonstrated industrial relevance (Bhardwaj et al., 2021; Zhang et al., 2024). Recent advances in metagenomics and strain engineering have further accelerated the discovery of novel, high-performance cellulases (Adab et al., 2024; Meng et al., 2025). Despite this progress, the high cost of enzyme production, low volumetric yields, and insufficient thermostability continue to restrict large-scale commercialization. Current strategies therefore focus on cost reduction through the exploitation of agro-industrial waste substrates, combined with systematic fermentation optimization and molecular strain improvement (Xiang et al., 2025).

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Animal waste, and cow dung in particular, represents an underutilized yet highly promising reservoir for cellulolytic microorganism discovery. As a direct by-product of ruminant digestion, cow dung contains partially degraded plant material that is inherently rich in cellulosic substrates and harbors a naturally diverse microbial community pre-adapted to cellulose degradation (Shaikh et al., 2025). Its exploitation as an enzyme source simultaneously provides an economically accessible substrate and contributes to sustainable waste management. The present study therefore aimed to isolate and characterize cellulase-producing bacteria from cow dung, to quantify their enzymatic output via secondary liquid-culture screening, and to establish their biochemical profiles as a foundation for future strain optimization and industrial deployment.

II. Materials And Methods

2.1 Sample Collection

Fresh cow dung samples were collected aseptically from a cowshed located near Shri Ram College, Muzaffarnagar, India, using sterile spatulas and pre-sterilized Petri dishes. Strict aseptic precautions were maintained throughout collection to prevent the introduction of extraneous environmental contaminants. Samples were transported to the laboratory in sealed sterile containers and processed on the day of collection to maximize microbial viability.

2.2 Serial Dilution And Primary Isolation

Serial dilution was employed to separate individual bacterial colonies from the complex mixed microbial population of cow dung. Approximately 1 g of the sample was suspended in 10 mL of sterile distilled water and vortexed to prepare the primary stock suspension. Sequential tenfold dilutions were prepared by transferring 1 mL of suspension into successive tubes each containing 9 mL of sterile distilled water, generating dilution gradients from 10^{-1} to 10^{-6} under aseptic conditions.

2.3 Nutrient Agar Medium Preparation And Isolation

Nutrient Agar Medium was prepared by dissolving peptone (0.5 g), beef extract (0.3 g), NaCl (0.5 g), and bacteriological agar (1.5 g) in 100 mL of distilled water. The medium was sterilized by autoclaving at 121°C and 15 psi (103.4 kPa) for 15 minutes. Following cooling to approximately 45–50°C within a laminar air flow (LAF) cabinet, the molten agar was aseptically dispensed into sterile Petri dishes and allowed to solidify. Aliquots of 0.1 mL from appropriate dilutions were spread uniformly onto the agar surface using a sterile glass spreader and the plates were incubated aerobically at 37°C for 24–48 h.

2.4 Morphological Characterization

Bacterial isolates were characterized morphologically using two staining approaches. Simple staining with methylene blue provided preliminary information on cell shape and spatial arrangement. Gram staining was subsequently performed using the standard four-step protocol: crystal violet (primary stain), Gram's iodine (mordant), 95% ethyl alcohol (decolorizer), and safranin (counterstain). The stained preparations were examined under oil-immersion light microscopy at 1,000× total magnification to determine Gram reaction and cellular morphology.

2.5 Selective Isolation Using Cmc Agar And Congo Red Assay

CMC agar medium was prepared using peptone (1 g), CMC (1 g), K_2HPO_4 (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.03 g), $(NH_4)_2SO_4$ (0.25 g), gelatin (0.2 g), and bacteriological agar (1 g) dissolved in 100 mL of distilled water, sterilized at 121°C for 15 minutes. The $MgSO_4$ and K_2HPO_4 components serve as buffering agents and provide essential Mg^{2+} cofactors necessary for cellulase catalytic activity. Pure bacterial colonies from nutrient agar plates were spot-inoculated onto CMC agar and incubated aerobically at 30–37°C for 3–7 days.

Following incubation, plates were flooded with a 1% (w/v) aqueous Congo red solution for 15 minutes. Congo red forms strong, non-covalent associations with intact β-1,4-D-glucopyranosyl polymers. The excess dye was removed by washing with 1 M NaCl, which selectively disrupts the weaker interactions between the dye and shorter, hydrolyzed oligosaccharides. Colonies that had secreted active endoglucanases produced visible, sharply defined, pale or completely clear halo zones of hydrolysis against the deeply pigmented red background. The diameter of each halo was measured in centimeters and used as a semi-quantitative index of cellulolytic potency.

2.6 Inoculum Development

Pure isolates were maintained on CMC agar slants at 4°C. For inoculum preparation, selected high-performing strains were cultivated in liquid broth containing $MgSO_4$ (0.03%), KH_2PO_4 (0.2%), glucose (1%), $(NH_4)_2SO_4$ (0.25%), and peptone (1%) at pH 7.0. Cultures were incubated at 37°C for 24 h on an orbital shaker to achieve late exponential (log) phase, maximizing cellular density and metabolic vigor prior to enzyme induction.

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2.7 Cellulase Enzyme Production

Cellulase production was conducted in a mineral salt medium containing CMC (5 g/L) as the primary carbon source and enzyme inducer, supplemented with $CaCl_2$ (0.005 g/L), $FeCl_2$ (0.00005 g/L), $ZnSO_4$ (0.005 g/L), $MnCl_2$ (0.000126 g/L), NH_4Cl (1 g/L), NaCl (1 g/L), $MgSO_4$ (0.82 g/L), KH_2PO_4 (1.25 g/L), and yeast extract (5 g/L), adjusted to pH 7.0. Inoculated Erlenmeyer flasks were incubated at 37°C for 48 h on an orbital shaker at 150 rpm. After incubation, culture broth was centrifuged at 14,000 × g for 30 minutes at 4°C, and the resulting cell-free supernatant was collected as the crude extracellular enzyme preparation.

2.8 Estimation Of Cellulase Activity (Dns Method)

Endoglucanase activity was quantified using the 3,5-dinitrosalicylic acid (DNS) colorimetric assay. The reaction mixture comprised 0.5 mL of crude enzyme supernatant and 0.5 mL of 1% (w/v) CMC in 0.05 M phosphate buffer (pH 8.0), incubated at 50°C for 30 minutes. The reaction was terminated by adding 1.5 mL of DNS reagent, followed by immersion in a boiling water bath (100°C) for 10 minutes. The DNS reagent is chemically reduced by liberated reducing sugars to 3-amino-5-nitrosalicylic acid, generating a proportional color shift from pale yellow to deep reddish-brown. Absorbance was measured spectrophotometrically at 540 nm, and reducing sugar concentration was calculated from a glucose standard calibration curve. One unit (U) of cellulase activity was defined as the quantity of enzyme releasing 1 µmol of reducing sugar equivalents (as glucose) per minute under the specified assay conditions.

2.9 Biochemical Characterization

To establish the physiological and metabolic profiles of the most active isolates, the following standardized biochemical assays were conducted:

Oxidase Test: Bacterial smears on filter paper impregnated with Kovács oxidase reagent were assessed for the development of dark purple colouration, confirming cytochrome c oxidase activity and an aerobic respiratory electron transport chain.

Catalase Test: A 3% hydrogen peroxide (H_2O_2) solution was applied directly to isolated colonies. Vigorous effervescence indicated catalase activity, confirming the organism's capacity to decompose reactive oxygen species into water and oxygen.

Indole Test: Isolates were cultured in tryptophan-rich broth at 37°C for 24–48 h. Addition of Kovács reagent yielded a pink or cherry-red ring at the broth surface in positive isolates, confirming tryptophanase activity and the catabolism of tryptophan to indole, pyruvate, and ammonia.

Methyl Red (MR) Test: Addition of methyl red indicator to MR-VP broth cultures detected the stable acid production characteristic of mixed-acid fermentation. A persistent red colouration indicated a positive result.

Voges–Proskauer (VP) Test: Sequential addition of Barritt's Reagents (α-naphthol and KOH) to MR-VP broth detected acetoin production from the butanediol fermentation pathway. A reddish-brown colouration indicated a positive result.

Citrate Utilization Test: Isolates were streaked onto Simmons citrate agar slants. A colour shift from forest green to Prussian blue, indicating medium alkalinization via citrate metabolism, confirmed the ability to utilize citrate as the sole carbon and energy source.

Triple Sugar Iron (TSI) Test: Inoculation into TSI agar (containing glucose, lactose, sucrose, phenol red, and ferrous sulfate) differentiated carbohydrate fermentation patterns and hydrogen sulfide production by color changes and black precipitate formation, respectively.

Sugar Fermentation Test: Growth in lactose broth with phenol red indicator and an inverted Durham tube assessed fermentative metabolism. Acid production (yellow colouration) and gas accumulation in the Durham tube indicated a positive result.

Urease Test: Cultivation in urea broth with phenol red indicator assessed urease enzyme activity. A shift to bright pink/fuchsia, indicating ammonia production and consequent medium alkalinization, indicated a positive result.

Hydrogen Sulfide Production Test: Isolates were grown in a sulfur-containing medium fortified with iron salts. Formation of a characteristic black iron sulfide precipitate confirmed H_2S production from sulfur-containing substrates.

III. Results

3.1 Isolation And Morphological Characterization

Aerobic cultivation of serially diluted cow dung samples on nutrient agar at 37°C yielded a morphologically diverse array of bacterial colonies following 24–48 h of incubation.

Colonies exhibited marked variability in size, shape, surface texture, margin characteristics, and pigmentation, reflecting successful recovery of a widely varied microbial population from the sample matrix. This macroscopic diversity confirmed that cow dung harbors a rich and complex bacterial community. Gram staining of all tested isolates confirmed that the dominant cellulolytic strains retained the crystal violet-iodine complex following alcohol decolorization, appearing as deeply pigmented purple cells under oil-immersion microscopy. This established their identity as **Gram-positive bacteria**. Cellular morphology was uniformly elongated and rod-shaped, consistent with the *Bacillus* morphotype, a genus widely reported as a prolific producer of extracellular cellulases.

3.2 Primary Screening: Congo Red ASSAY

Primary screening on CMC agar plates, followed by Congo red dye flooding and 1 M NaCl destaining, revealed distinct clear halos of hydrolysis surrounding cellulolytically active colonies, set against the deeply red-stained agar background. The diameter of hydrolysis halos varied considerably across the twenty isolates tested, directly reflecting differences in extracellular endoglucanase secretion capacity. Table 1 presents the measured hydrolysis zone data for all isolates.

Table 1.
Zone of CMC Hydrolysis of Bacterial Isolates from Cow Dung (Congo Red Assay). ★ denotes isolates selected for further characterization.

S.No	Isolate No.	Zone of Hydrolysis (cm)
1	SRCMBK11	1.0 ★
2	SRCMBK12	0.5
3	SRCMBK13	0.4
4	SRCMBK14	0.2
5	SRCMBK15	0.3
6	SRCMBK16	0.5
7	SRCMBK17	0.4
8	SRCMBK18	0.6
9	SRCMBK19	0.2
10	SRCMBK110	1.1 ★
11	SRCMBK111	0.3
12	SRCMBK112	0.3
13	SRCMBK21	0.9
14	SRCMBK22	1.0 ★
15	SRCMBK23	1.1 ★
16	SRCMBK24	0.5
17	SRCMBK25	1.0
18	SRCMBK26	0.7
19	SRCMBK27	0.5
20	SRCMBK28	1.2 ★

Note: Zone diameters measured as the diameter (cm) of the hydrolysis halo following Congo red flooding and 1 M NaCl destaining. Values represent single-replicate primary screening measurements.

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Five isolates — SRCMBK28, SRCMBK110, SRCMBK23, SRCMBK11, and SRCMBK22 — produced hydrolysis zones of ≥1.0 cm and were selected as elite producers for secondary screening and detailed biochemical profiling. Isolate SRCMBK28 produced the largest halo (1.2 cm), followed by SRCMBK110 and SRCMBK23 (both 1.1 cm). The remaining isolates produced zones ranging from 0.2 cm (SRCMBK14, SRCMBK19) to 0.9 cm (SRCMBK21), indicating a wide spectrum of endoglucanase secretion capacity within the cow dung microbiome.

3.3 Glucose Standard Curve

A glucose standard calibration curve was constructed by measuring absorbance at 540 nm across a series of known glucose concentrations. The curve demonstrated a strong linear relationship between absorbance and glucose concentration, confirming the validity of the DNS colorimetric method for quantifying reducing sugars released during enzymatic hydrolysis. Table 2 presents the calibration data used in all subsequent cellulase activity calculations.

Table 2.
Glucose Standard Curve Data for DNS Colorimetric Cellulase Activity Assay (Absorbance at 540 nm).

Glucose Concentration (mg/mL)	Absorbance at 540 nm
0.0	0.000
0.2	0.053
0.4	0.108
0.6	0.162
0.8	0.218
1.0	0.271
1.2	0.298

Note: Values represent mean absorbance readings from triplicate measurements of freshly prepared glucose standards.

3.4 Secondary Screening: Enzyme Production Kinetics

Quantitative analysis of cellulase production in liquid mineral salt medium revealed a characteristic growth-associated enzyme production profile across all elite isolates. During the initial lag phase (approximately Days 1–2), minimal extracellular enzyme activity was detected as bacterial populations adapted metabolically to the culture medium. This was followed by a rapid and pronounced increase in cellulase activity throughout the exponential (log) phase (Days 2–4), driven by active cellular proliferation and accelerating substrate utilization. Peak cellulase activity, as quantified by absorbance at 540 nm, was recorded during the late exponential to early stationary phase (approximately Days 4–6), corresponding to the period of maximum cellular biomass and heightened secretion of extracellular enzymes in response to nutrient limitation signals. During the stationary phase (Days 5–6), activity plateaued as nutrient depletion and the accumulation of metabolic by-products constrained further microbial growth. A subsequent decline phase was observed from Day 7 onward, attributed to reduced cellular viability and potential protease-mediated degradation of secreted cellulase proteins. These kinetic observations confirm that the late exponential to early stationary phase constitutes the optimal harvesting window for maximum cellulase yield from these isolates.

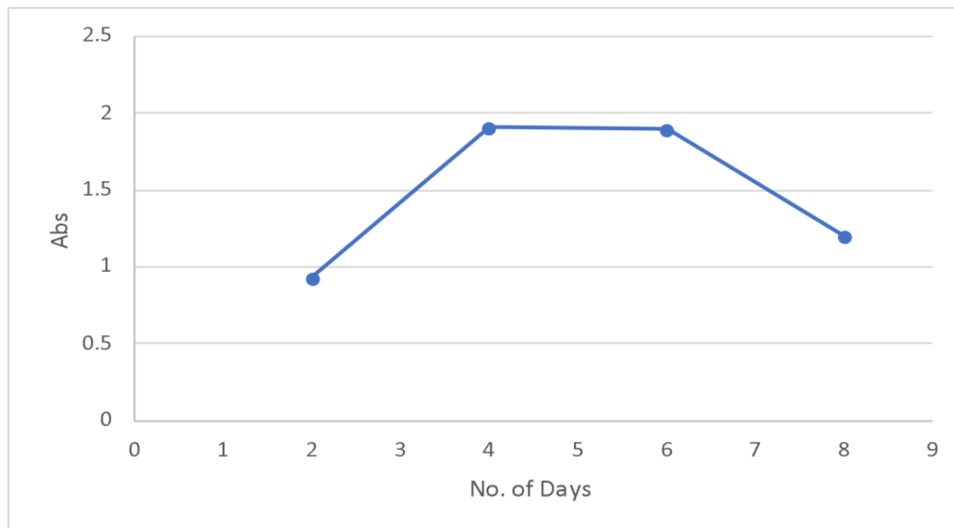

Figure : Cellulase Activity Profile — absorbance at 540 nm plotted against time (days) for selected elite isolates, demonstrating peak activity at the late exponential to early stationary growth phase.

3.5 Biochemical Characterization

Comprehensive biochemical profiling of the five elite isolates (SRCMBK28, SRCMBK110, SRCMBK23, SRCMBK11, and SRCMBK22) revealed a consistent and broadly positive metabolic phenotype across all assays. The cumulative results are summarized in Table 3.

Table 3.
Biochemical Characterization Profile of Elite Cellulolytic Isolates from Cow Dung.

Biochemical Test	Reagent / Indicator	Positive Result Criteria	Observed Result (Top Isolates)
Oxidase	Kovács oxidase reagent on filter paper	Dark purple colouration	Positive (+)
Catalase	3% H_2O_2 applied directly to colonies	Vigorous O_2 effervescence (bubbles)	Positive (+)
Indole	Kovács reagent; tryptophan broth, 37°C, 24–48 h	Pink/cherry-red ring at broth surface	Positive (+)
Methyl Red (MR)	Methyl red indicator in MR-VP broth	Stable red colouration (mixed-acid fermentation)	Positive (+)
Voges–Proskauer (VP)	Barritt's Reagents (α-naphthol + KOH)	Reddish-brown colouration (acetoin)	Positive (+)
Citrate Utilization	Simmons citrate agar (bromothymol blue)	Colour shift: green → blue (alkalinization)	Positive (+)
Triple Sugar Iron (TSI)	Phenol red + ferrous sulfate in TSI agar	Red slant (alkaline); orange butt (neutral)	Positive (+)
Sugar Fermentation	Phenol red broth + Durham tube (lactose)	Yellow medium; gas in Durham tube	Positive (+)
Urease	Phenol red in buffered urea broth	Bright pink/fuchsia (ammonia production)	Positive (+)
H_2S Production	Iron salt–supplemented agar	Black iron sulfide precipitate	Positive (+)

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All five isolates yielded positive oxidase and catalase reactions, confirming aerobic respiratory metabolism and active protection against oxidative stress. Positive indole and methyl red results indicate the capacity for tryptophan catabolism and mixed-acid glucose fermentation, respectively. Positive Voges–Proskauer results confirm the ability to produce acetoin via the butanediol pathway. Citrate utilization, carbohydrate fermentation (confirmed by TSI and lactose broth tests), urease activity, and hydrogen sulfide production were uniformly positive across the panel, collectively confirming the broad substrate versatility and metabolic adaptability of these isolates.

IV. Discussion

4.1 Taxonomic Inference

The consistent combination of Gram-positive, rod-shaped morphology, positive catalase and oxidase activity, citrate utilization, and robust extracellular enzyme secretion constitutes a biochemical profile closely aligned with the genus *Bacillus*. Members of this genus, particularly *B. subtilis*, *B. licheniformis*, and *B. pumilus*, are widely documented in the literature as high-yield producers of extracellular cellulases and hemicellulases with significant industrial utility (Bhardwaj et al., 2021). Their successful isolation from cow dung is consistent with prior reports confirming *Bacillus* species as numerically dominant and enzymatically active members of herbivore gut and dung microbial communities (Shaikh et al., 2025). However, definitive taxonomic identification based on phenotypic biochemical assays alone is a recognized limitation of classical microbiology. Modern microbiological standards require 16S rRNA gene sequencing, or ideally whole-genome sequencing, for unambiguous species-level classification. Without molecular data, the precise identity, potential pathogenicity classification, and intellectual property status of the SRCMBK isolates remain unresolved. Molecular identification is therefore designated as an indispensable next step.

4.2 Substrate Limitation Of Cmc-Based Screening

A critical limitation of the primary screening methodology employed in this study concerns the nature of the substrate used. CMC is a highly soluble, chemically modified, and entirely amorphous derivative of native cellulose. Due to the absence of a recalcitrant crystalline structure, CMC is readily hydrolyzed by endoglucanases alone, without the requirement for a complete, synergistic cellulase complex.

The Congo red halo assay therefore measures endoglucanase activity exclusively and does not confirm the presence of cellobiohydrolases (exoglucanases) or β-glucosidases — the two other enzyme classes essential for the complete deconstruction of crystalline, native lignocellulose. Real-world industrial feedstocks, such as rice straw, wheat bran, or sugarcane bagasse, are predominantly crystalline and intimately intertwined with protective lignin. Strains performing well on CMC agar commonly exhibit substantially reduced activity when challenged with these native substrates. The impressive 1.2 cm hydrolysis zone produced by SRCMBK28 therefore reflects strong endoglucanase secretion, but cannot serve as a proxy for complete cellulolytic capability on industrial biomass. Future investigations must validate these isolates against crystalline substrates (e.g., Avicel PH-101 or Whatman No. 1 filter paper) and untreated lignocellulosic agricultural residues before any conclusion regarding industrial viability can be drawn.

4.3 Enzyme Production Kinetics And Bioprocess Implications

The growth-associated enzyme production profile observed in this study — with peak cellulase activity at the late exponential to early stationary phase — is biochemically consistent with established models of inducible extracellular enzyme secretion in facultative aerobes. As cellular growth rate begins to decelerate due to nutrient limitation, bacteria upregulate the expression of scavenging enzymes, including cellulases, to access complex exogenous carbon sources. This kinetic pattern provides a highly predictable and practically accessible harvesting window, facilitating straightforward optimization of harvest timing in scaled fermentation systems. From a bioprocess engineering perspective, the use of CMC as the sole carbon source in the production medium is biochemically rational as a proof of concept but is economically unsustainable at industrial scale. Transitioning to inexpensive, lignocellulose-rich agro-industrial residues — such as wheat bran, corn cob, or rice husk — as alternative carbon substrates and physical support matrices in solid-state fermentation (SSF) systems would dramatically reduce production costs. SSF has been widely reported to enhance cellulase yield from *Bacillus* strains relative to submerged fermentation, owing to the natural affinity of these organisms for solid substrates and the higher substrate surface-to-volume ratios achievable in SSF configurations (Xiang et al., 2025).

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4.4 Industrial Relevance And Future Perspectives

The identification of multiple potent cellulolytic isolates from cow dung supports the broader premise that ruminant waste constitutes an accessible and economically attractive bioprospecting resource. Beyond its application in biofuel production, cellulase enzymes derived from these strains could find utility in textile biopolishing, pulp and paper processing, animal feed digestibility enhancement, and food processing applications — all sectors in which cellulases are classified as industrially critical (Hussain et al., 2025). To fully translate these preliminary findings into commercial applications, future research should prioritize: (1) definitive molecular identification via 16S rRNA gene sequencing; (2) rigorous validation of cellulolytic activity against crystalline cellulose and untreated lignocellulosic substrates; (3) systematic fermentation optimization using Response Surface Methodology (RSM) to maximize enzyme titer, thermostability, and pH tolerance; and (4) protein purification and biochemical characterization of the secreted cellulases to establish kinetic parameters (Km, Vmax) essential for rational industrial process design.

V. Conclusion

This study successfully demonstrated the isolation, primary and secondary screening, and comprehensive biochemical characterization of cellulase-producing bacteria from cow dung. Selective CMC agar screening using the Congo red dye displacement assay identified five elite isolates — SRCMBK28, SRCMBK110, SRCMBK23, SRCMBK11, and SRCMBK22 — on the basis of superior hydrolysis zone diameters (≥1.0 cm). Gram staining and biochemical profiling consistently characterized the dominant active isolates as Gram-positive, rod-shaped bacteria with a broad metabolic profile, strongly suggestive of the genus *Bacillus*. Secondary liquid-culture screening confirmed a growth-associated cellulase production profile, with peak endoglucanase activity quantified during the late exponential to early stationary growth phase, providing a clearly defined and reproducible harvesting window. The study acknowledges the inherent methodological limitation of using amorphous CMC as the sole screening substrate, which selectively measures endoglucanase activity and does not confirm the complete cellulase complex activity required for native crystalline biomass deconstruction.

Advancing these isolates toward commercial viability therefore necessitates validation against crystalline cellulose substrates, molecular taxonomic identification, and systematic bioprocess optimization. Overall, the findings confirm cow dung as a rich, accessible, and economically viable source of industrially relevant cellulolytic bacteria, contributing a robust foundation for the development of sustainable enzyme-based biomass conversion strategies.

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