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Screening and Identification of Cellulolytic Bacteria from Goat Gastrointestinal Tract for Enhanced Enzyme Production

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Abstract-- **The present study reports the isolation, selective screening, and biochemical characterization of cellulase-producing bacteria sourced from the gastrointestinal tract (rumen) of goats (*Capra hircus*). The ruminant rumen constitutes one of the most evolutionarily refined, lignocellulose-degrading ecosystems known to biology, harboring a dense and diverse microbial consortium. Rumen contents were collected under strict aseptic conditions, subjected to serial dilution, and cultivated on nutrient agar medium under aerobic conditions. Resulting bacterial colonies were screened for cellulolytic activity on Carboxymethyl Cellulose (CMC) agar using a Congo red dye displacement assay. Clear zones of hydrolysis around colonies confirmed the extracellular secretion of endoglucanases. Gram staining revealed that the dominant cellulolytic isolates were Gram-positive, rod-shaped bacteria. Quantitative analysis of hydrolysis zones across sixteen isolates identified strains SRCMBA23, SRCMBA18, and SRCMBA19 as the most active producers, with zone diameters of 1.3 cm, 1.2 cm, and 1.1 cm, respectively. A comprehensive battery of standardized biochemical assays confirmed the metabolic versatility of these elite strains, including positive results for oxidase, catalase, indole production, methyl red, citrate utilization, urease activity, carbohydrate fermentation, and hydrogen sulfide production. The taxonomic profile strongly suggests affiliation with the genus *Bacillus*. The study acknowledges the inherent methodological limitation of aerobic cultivation applied to an obligate anaerobic ecosystem, and discusses the advantage this confers for industrial scale-up. The findings establish the caprine rumen as a promising biological reservoir of robust cellulolytic bacteria with significant potential for biofuel production, lignocellulosic waste valorization, and industrial enzyme applications. Molecular identification and fermentation optimization are recommended as essential next steps.**

Keywords--**Cellulase; goat rumen; Carboxymethyl Cellulose (CMC); cellulolytic bacteria; Congo red assay; biochemical characterization; lignocellulosic biomass; Bacillus; bioethanol**

I. INTRODUCTION

Lignocellulosic biomass, derived principally from agricultural residues, forestry by-products, and dedicated bioenergy crops, represents the most abundant and renewable organic substrate on Earth. Global annual production is estimated to exceed 120 billion metric tons, presenting a substantial opportunity to advance the transition from fossil-fuel-dependent economies toward sustainable, bio-based production systems (Bhardwaj et al., 2021). However, the structural recalcitrance of lignocellulose severely limits its direct bioconversion. The material comprises an interwoven matrix of three primary macromolecules—cellulose, hemicellulose, and lignin. Cellulose itself is a linear homopolymer of β-(1→4)-linked D-glucopyranose units; extensive intra- and intermolecular hydrogen bonding confers a highly ordered crystalline architecture that renders the substrate exceptionally resistant to both chemical depolymerization and biological degradation.

Efficient hydrolysis of cellulose requires the synergistic action of a tripartite enzyme system collectively designated as cellulases. Endoglucanases (EC 3.2.1.4) randomly cleave internal β-1,4 glycosidic bonds within amorphous regions of cellulose chains, generating numerous new reducing and non-reducing chain ends. Cellobiohydrolases (exoglucanases; EC 3.2.1.91) act processively from these chain ends to liberate the disaccharide cellobiose. β-Glucosidases (EC 3.2.1.21) subsequently hydrolyze cellobiose and short cello-oligosaccharides into fermentable glucose, thereby relieving end-product inhibition of the upstream enzymes. Many cellulases feature a bipartite architecture comprising a catalytic domain connected via a flexible, glycosylated linker peptide to a carbohydrate-binding module (CBM), which maintains enzyme proximity to the insoluble substrate and dramatically enhances catalytic efficiency (Zhang et al., 2024). Cellulase systems are broadly classified as non-complexed, freely secreted systems (typical of aerobic fungi and bacteria) or multi-enzyme cellulosomes (characteristic of anaerobic bacteria) (Bhardwaj et al., 2021). The global industrial demand for thermostable and efficient microbial cellulases has grown considerably over the past decade. In the bioenergy sector, cellulases are central to second-generation bioethanol production, enabling saccharification of pretreated agricultural residues into fermentable sugars.

Beyond bioenergy, cellulases are widely deployed in textile biopolishing and denim biostoning, pulp and paper bio-bleaching and de-inking, fruit juice clarification, and as feed additives to improve the digestibility of fibrous animal feeds. Cellulases currently rank among the top three commercially significant industrial enzyme categories worldwide, driving continued bioprospecting for novel, high-yielding microbial strains (Hussain et al., 2025; Ezea, 2025). Among the diverse natural habitats harboring cellulolytic microorganisms, the ruminant gastrointestinal tract—specifically the reticulorumen—represents one of the most efficient lignocellulose-degrading bioreactors known to biology. The rumen maintains a constant physiological temperature of approximately 39°C, an oxygen-depleted environment with an oxidation-reduction potential below −300 mV, and a pH range of 6.0 – 7.0. These conditions sustain a dense microbial consortium—exceeding 10^{10} bacterial cells per milliliter of ruminal fluid—comprising obligate anaerobic bacteria, ciliate protozoa, anaerobic chytridiomycete fungi, and methanogenic archaea. Goats (*Capra hircus*), as highly adaptable browsers consuming a diverse and frequently lignified diet of shrubs, tree bark, and coarse grasses, likely harbor particularly robust and potent fibrolytic strains adapted to severe substrate recalcitrance.

A recognized limitation in exploiting the rumen's enzymatic potential is the "great plate count anomaly": an estimated 70–80% of ruminal microorganisms are obligate anaerobes that are currently unculturable using standard laboratory techniques. The keystone cellulolytic species responsible for the majority of *in vivo* fiber digestion—such as *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*—require technically demanding, strictly anaerobic cultivation methods such as the Hungate roll-tube technique. Conversely, aerobic isolation protocols selectively recover facultative anaerobes and endospore-forming aerobes (principally *Bacillus* spp.), which, while representing a minor fraction of the *in vivo* rumen population, offer significant practical advantages for industrial applications: rapid growth kinetics, robust extracellular enzyme secretion, extreme environmental tolerance via endospore formation, and amenability to large-scale aerobic submerged fermentation (Seo et al., 2013).

Recent advances in functional metagenomics (Adab et al., 2024) and CRISPR-Cas9-mediated strain engineering (Meng et al., 2025) are further expanding the frontier of cellulase discovery and optimization.

In alignment with these imperatives, the present study details the primary isolation, differential screening, and comprehensive biochemical characterization of cellulase-producing bacteria from the goat gastrointestinal tract, employing established culture-dependent aerobic methodologies. The study also critically evaluates the inherent methodological limitations of the aerobic approach, the biochemical basis for differential bacterial susceptibility, and the techno-economic implications for industrial enzyme production.

II. MATERIALS AND METHODS

2.1 Sample Collection And Preservation

Rumen contents (comprising partially digested, fibrous plant material) were collected directly from the gastrointestinal tract of a freshly slaughtered goat under strict aseptic conditions, to preserve the native microbial community and prevent the introduction of environmental contaminants. The collected material was immediately transferred to sterile, hermetically sealed, wide-mouth containers and transported to the laboratory under controlled temperature conditions. To maximize the metabolic viability of the sensitive ruminal microflora, all cultivation procedures commenced on the same day as sample procurement.

2.2 Serial Dilution And Primary Isolation

To isolate discrete bacterial colonies from the densely populated rumen sample, a standard serial dilution technique was employed. Approximately 1 g of raw rumen material was aseptically homogenized in 10 mL of sterile distilled water to prepare the primary stock suspension. Following vigorous vortexing to dislodge adherent bacterial cells from the particulate matrix, sequential 10-fold dilutions were prepared by transferring 1 mL of suspension into successive tubes each containing 9 mL of sterile distilled water. This process was repeated to achieve dilution gradients of 10^{-4} to 10^{-6}, ensuring statistically countable and physically separated colonies upon agar plating.

2.3 Preparation Of Nutrient Agar Medium

Nutrient Agar Medium (NAM) was prepared using peptone (0.5 g), beef extract (0.3 g), NaCl (0.5 g), and bacteriological agar (1.5 g) dissolved in 100 mL of distilled water. The medium was sterilized by autoclaving at 121°C and 15 psi (103.4 kPa) for 15 minutes. After 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 each dilution were spread uniformly using a sterile glass spreader, and the inoculated plates were incubated aerobically at 37°C for 24–48 h.

2.4 Selective Isolation Of Cellulolytic Bacteria Using Cmc Agar

Carboxymethyl Cellulose (CMC) agar 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 inclusion of $MgSO_4$ and K_2HPO_4 serves to buffer medium pH and provide Mg^{2+} cofactors essential for cellulase catalytic activity. Pure bacterial colonies selected from nutrient agar plates were spot-inoculated onto CMC agar and incubated aerobically at 37°C for 48 h.

2.5 Congo Red Dye Displacement Assay

Following the 48-hour incubation, CMC agar 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 then removed by washing with 1 M NaCl, which disrupts the weaker interactions between the dye and shorter, hydrolyzed oligosaccharides. Colonies that had secreted active endoglucanases generated distinct, clearly defined halo zones of hydrolysis against the deeply pigmented red background. The diameter of each halo was measured in centimeters using a calibrated ruler, providing a semi-quantitative index of cellulolytic potency.

2.6 Morphological Characterization (Gram Staining)

The cellular morphology and Gram reaction of the most active cellulolytic isolates were determined using the standard Gram staining protocol. Thin smears of pure cultures were prepared on clean glass slides, heat-fixed, and subjected to sequential treatment with crystal violet (primary stain), Gram's iodine (mordant), 95% ethyl alcohol (decolorizer), and safranin (counterstain). Slides were examined under oil-immersion light microscopy at 1,000× total magnification.

2.7 Inoculum Preparation And Secondary Screening

Pure cultures were maintained on CMC agar slants at 4°C. For inoculum development, selected high-performing isolates were transferred aseptically into 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, and incubated at 37°C for 24 h on an orbital shaker. This duration was selected to harvest cultures at late exponential (log) phase, maximizing cellular density and metabolic activity prior to enzyme induction.

2.8 Cellulase Enzyme Production

Cellulase production was carried out 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. The resulting cell-free supernatant was designated as the crude extracellular enzyme preparation.

2.9 Estimation Of Cellulase Activity (Dns Method)

Endoglucanase activity was quantified using the 3,5-dinitrosalicylic acid (DNS) colorimetric method. The reaction mixture comprised 0.5 mL 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 addition of 1.5 mL DNS reagent, followed by immersion in a boiling water bath (100°C) for 10 minutes. The DNS reagent is reduced by liberated reducing sugars (glucose and cellobiose) to 3-amino-5-nitrosalicylic acid, producing a color shift from pale yellow to deep reddish-brown proportional to reducing sugar concentration. Absorbance was measured spectrophotometrically at 540 nm, and reducing sugar concentrations were 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 defined assay conditions.

2.10 Biochemical Characterization

To determine the physiological and metabolic profiles of the most active cellulolytic isolates, the following standardized biochemical tests were performed:

Oxidase Test: Bacterial smears on filter paper were exposed to Kovács oxidase reagent. Development of a dark purple colouration indicated a positive result, confirming the presence of cytochrome c oxidase and an aerobic respiratory chain.

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

Indole Test: Isolates were cultivated in tryptophan-rich broth for 24–48 h. Addition of Kovács reagent produced a pink or cherry-red ring at the broth surface in positive results, indicating the activity of tryptophanase and the catabolism of tryptophan to indole, pyruvate, and ammonia.

Methyl Red (Mr) Test: After growth in MR-VP broth, addition of methyl red indicator detected stable acid production characteristic of mixed-acid fermentation. A red colour indicated a positive result.

Voges–Proskauer (Vp) Test: Sequential addition of Barritt's Reagents (α-naphthol and KOH) to MR-VP broth tested for acetoin production from the butanediol fermentation pathway. A reddish-pink colour 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, confirmed the ability to utilize citrate as the sole carbon and energy source via citrate permease.

Triple Sugar Iron (Tsi) Test: Inoculation into TSI agar (containing glucose, lactose, sucrose, phenol red, and ferrous sulfate) assessed complex carbohydrate fermentation patterns and hydrogen sulfide (H_2S) production, as indicated 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 fermentation of specific carbohydrates. Acid production (yellow color) and gas accumulation in the Durham tube indicated positive results.

Urease Test: Cultivation in urea broth containing phenol red indicator assessed urease activity. A shift to bright pink/fuchsia indicated ammonia production from urea hydrolysis and consequent medium alkalinization.

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

III. RESULTS

3.1 Primary Isolation And Morphological Characterization

Primary aerobic cultivation of serially diluted goat rumen samples on nutrient agar at 37°C yielded a diverse and morphologically heterogeneous bacterial consortium following 24–48 h of incubation.

Colonies exhibited marked variability in diameter, shape, margin characteristics (smooth to undulate), and elevation, reflecting successful recovery of a varied microbial population from the complex rumen matrix.

Gram staining of the most active isolates revealed that all targeted strains retained the crystal violet-iodine complex following alcohol decolorization, appearing as deeply pigmented purple cells under oil-immersion microscopy. This confirmed their identity as **Gram-positive bacteria**. Cellular morphology was uniformly that of elongated, rod-shaped structures (bacillus morphotype), consistent with members of the genus *Bacillus*.

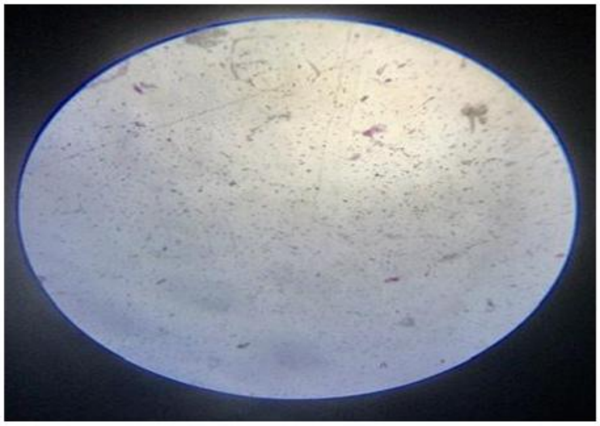

Figure 1: Gram staining of bacterial isolates showing Gram-positive rod-shaped cells.

3.2 Cellulolytic Screening: Congo Red Assay

Transfer of pure colonies to selective CMC agar followed by Congo red dye displacement revealed clear, sharply defined halo zones of hydrolysis around cellulolytically active colonies, set against a deeply pigmented red background. The diameter of these hydrolysis halos varied considerably among isolates, directly reflecting differences in endoglucanase secretion capacity. Measured zone diameters for all sixteen isolates are presented in Table 1.

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

S.No	Isolate No.	Zone of Hydrolysis (cm)
1	SRCMBA11	0.3
2	SRCMBA12	0.2
3	SRCMBA13	0.6
4	SRCMBA14	0.5
5	SRCMBA15	1.0
6	SRCMBA16	0.9
7	SRCMBA17	0.6
8	SRCMBA18	1.2 ★
9	SRCMBA19	1.1 ★
10	SRCMBA20	0.9
11	SRCMBA21	0.7
12	SRCMBA22	0.6
13	SRCMBA23	1.3 ★
14	SRCMBA24	0.6
15	SRCMBA25	0.5
16	SRCMBA26	0.5

Note: Zones measured as diameter (cm) of hydrolysis halo following Congo red flooding and 1 M NaCl wash. All measurements represent single replicate readings from primary screening plates.

Isolate SRCMBA23 produced the largest hydrolysis zone (1.3 cm), followed by SRCMBA18 (1.2 cm), SRCMBA19 (1.1 cm), and SRCMBA15 (1.0 cm). These four isolates were selected as elite producers for secondary screening and detailed biochemical characterization. Isolates SRCMBA11 and SRCMBA12 produced the smallest zones (0.3 cm and 0.2 cm, respectively), indicating minimal endoglucanase secretion under the screening conditions.

3.3 Biochemical Characterization

The comprehensive biochemical profile of the three most active isolates (SRCMBA23, SRCMBA18, and SRCMBA19) is summarized in Table 2. All three isolates produced consistent results across the panel of assays, indicating a uniform metabolic phenotype.

Table 2.
Biochemical Characterization Profile of Elite Cellulolytic Isolates (SRCMBA23, SRCMBA18, SRCMBA19).

Biochemical Test	Reagent / Indicator	Positive Result Criteria	Observed Result (SRCMBA23, 18, 19)
Oxidase	Kovács oxidase reagent	Dark purple colouration	Positive (+)
Catalase	3% H_2O_2 solution	Vigorous effervescence (O_2 bubbles)	Positive (+)
Indole	Kovács reagent after tryptophan broth	Pink/cherry-red ring at interface	Positive (+)
Methyl Red (MR)	Methyl red pH indicator	Stable red colouration	Positive (+)
Voges–Proskauer (VP)	Barritt's Reagents (α-naphthol + KOH)	Reddish-pink colouration	Positive (+)
Citrate Utilization	Simmons citrate agar (bromothymol blue)	Colour shift: green → blue	Positive (+)
Triple Sugar Iron (TSI)	Phenol red + ferrous sulfate	Alkaline slant (red); neutral butt (orange)	Positive (+)
Sugar Fermentation	Phenol red broth + Durham tube (lactose)	Yellow medium; gas in Durham tube	Positive (+)
Urease	Phenol red in urea broth	Bright pink/fuchsia colouration	Positive (+)
H_2S Production	Iron salt–containing agar	Black iron sulfide precipitate	Positive (+)

All three isolates were positive for oxidase and catalase, confirming an aerobic respiratory metabolism and enzymatic protection against oxidative stress. Positive indole and methyl red results indicate the capacity for tryptophan catabolism and mixed-acid glucose fermentation, respectively.

Citrate utilization, positive urease activity, and carbohydrate fermentation (confirmed by the TSI and lactose broth tests) collectively indicate broad metabolic versatility. Hydrogen sulfide production was also confirmed by the formation of a distinct black iron sulfide precipitate.

IV. DISCUSSION

4.1 Taxonomic Inferences From Biochemical And Morphological Data

The combination of Gram-positive rod morphology, spore-forming potential, robust catalase and oxidase activity, citrate utilization, and proficient extracellular enzyme secretion collectively aligns with the classical physiological hallmarks of the genus *Bacillus*. Species such as *B. subtilis*, *B. licheniformis*, and *B. pumilus* are well-documented producers of extracellular cellulases, xylanases, and hemicellulases and have been previously isolated from ruminant digestive tracts (Seo et al., 2013). Their successful recovery from goat rumen contents is consistent with the literature describing aerobically cultivable endospore-formers as a transient but enzymatically active minority of the rumen population, frequently ingested via soil-contaminated forage.

However, certain aspects of the biochemical profile merit critical attention. Canonical *Bacillus subtilis* is characteristically indole-negative and does not typically produce H_2S. The co-occurrence of indole positivity and H_2S production with otherwise typical *Bacillus* characteristics in the present isolates is atypical and warrants further investigation. This discrepancy may reflect either a novel, rumen-adapted variant that has acquired atypical metabolic traits under evolutionary pressure, or the inadvertent characterization of a tightly associated mixed-cell consortium that resisted complete separation during serial dilution. Definitive identification by 16S rRNA gene sequencing is essential to resolve this ambiguity.

4.2 Critical Methodological Evaluation: The Aerobic Isolation Paradox

A fundamental tension exists between the strictly anaerobic nature of the rumen ecosystem and the aerobic cultivation methodology employed in this study. The dominant fibrolytic species responsible for the majority of *in vivo* cellulose digestion—including *Fibrobacter succinogenes*, *Ruminococcus albus*, and the anaerobic fungus *Neocallimastix* spp.—are obligate anaerobes that cannot survive aerobic cultivation conditions. Consequently, the aerobic protocol employed here acts as a selective bottleneck, excluding the ecologically dominant cellulolytic species and enriching for facultative and obligate aerobic endospore-formers. This means the isolated strains do not accurately represent the *in vivo* fiber-degrading community of the goat rumen. Paradoxically, however, this same methodological limitation yields significant practical advantages. Obligate anaerobes are notoriously difficult and expensive to maintain at industrial scale.

The aerobically cultivable strains isolated here are substantially more amenable to the oxygenated, high-shear environments of commercial submerged fermentation bioreactors, greatly simplifying downstream industrial deployment.

4.3 Limitation Of CMC As A Screening Substrate

A second critical limitation concerns the use of CMC as the primary substrate for cellulolytic screening. CMC is a highly soluble, chemically modified, amorphous derivative of native cellulose that lacks the recalcitrant crystalline structure of true lignocellulosic biomass. The Congo red assay measures endoglucanase activity specifically; it does not assess the presence of cellobiohydrolases (exoglucanases) or the complete synergistic cellulase complex required for the efficient deconstruction of crystalline cellulose. Microorganisms that perform well on CMC agar frequently exhibit markedly reduced activity against crystalline substrates such as Avicel or Whatman filter paper (Béguin, Institut Pasteur). Therefore, while the 1.3 cm zone produced by isolate SRCMBA23 is indicative of robust endoglucanase secretion, future studies must validate these isolates against native crystalline cellulose and untreated lignocellulosic biomass to confirm their commercial viability.

4.4 Techno-Economic Considerations For Scale-Up

The transition from bench-scale characterization to commercial enzyme production remains constrained by significant techno-economic barriers. Cellulase production costs can account for as much as 40–48% of total downstream processing expenses in lignocellulosic biorefineries, largely due to low enzyme yields, slow hydrolysis kinetics, and severe end-product inhibition by cellobiose and glucose (Xiang et al., 2025). To reduce production costs, the global biotechnology industry has increasingly pivoted toward solid-state fermentation (SSF) strategies, utilizing cheap, abundant agricultural residues (wheat bran, sugarcane bagasse) as combined support matrices and carbon sources. Advanced bioprocess optimization using Response Surface Methodology (RSM) is also recognized as essential for maximizing enzyme titers (Shaikh et al., 2025).

4.5 Safety, Toxicity, And Regulatory Profile

Microbial cellulases are inherently biodegradable, non-toxic proteins that have been classified as Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) following rigorous 90-day repeated-dose oral toxicity studies in mammalian models.

This biocompatibility supports their use as direct-fed microbial additives in the animal feed industry and in food processing applications. However, broader industrial deployments—particularly in the textile sector—require attention to the toxicological profiles of associated co-solvents and dyes used in biopolishing and biostoning processes. Additionally, recent life-cycle risk assessments have identified inhalation of cellulose nanomaterials in immobilized enzyme manufacturing as a potential occupational hazard, necessitating comprehensive safety frameworks that address the holistic industrial process rather than the enzyme alone.

4.6 Future Perspectives: Molecular Identification, Metagenomics, And Synthetic Biology

Definitive taxonomic identification of the isolated strains by 16S rRNA gene sequencing and whole-genome sequencing is a critical next step, as phenotypic biochemical profiling alone is insufficient for unambiguous classification. Beyond culture-dependent approaches, functional and shotgun metagenomics offer transformative potential for discovering novel, hyper-active cellulases from the unculturable fraction of the rumen microbiome—a resource that current plate-based methods cannot access (Adab et al., 2024). Concurrently, CRISPR-Cas9-mediated genetic engineering of promising isolates—such as the targeted deletion of catabolite repression genes and overexpression of key transcriptional activators—can further optimize cellulase yield and thermal stability (Meng et al., 2025), accelerating the development of competitive industrial cell factories.

V. CONCLUSION

This study successfully demonstrated the isolation and biochemical characterization of cellulase-producing bacteria from goat gastrointestinal (rumen) samples. Selective screening using CMC agar and the Congo red dye displacement assay confirmed the cellulolytic activity of sixteen isolates, among which SRCMBA23 (1.3 cm), SRCMBA18 (1.2 cm), and SRCMBA19 (1.1 cm) exhibited the highest endoglucanase activity. Morphological analysis identified the dominant isolates as Gram-positive, rod-shaped bacteria, and the comprehensive biochemical profile strongly suggests affiliation with the genus *Bacillus*. The study critically acknowledges that the aerobic isolation methodology selectively excludes the dominant obligate anaerobic cellulolytic species of the native rumen ecosystem; however, the recovered aerobic strains offer substantial practical advantages for industrial-scale fermentation.

The goat rumen is confirmed as a rich and largely underexplored reservoir of cellulolytic bacteria with considerable potential for biofuel production, lignocellulosic waste valorization, and diverse industrial enzyme applications. Advancing these isolates toward commercial utility requires: (1) definitive molecular identification via 16S rRNA gene sequencing; (2) validation of cellulolytic activity against crystalline cellulose and native lignocellulosic biomass; (3) systematic optimization of fermentation parameters (temperature, pH, carbon-to-nitrogen ratio, agitation) to maximize cellulase titers; and (4) integration of metagenomic and synthetic biology approaches to access and engineer the full enzymatic potential of the caprine rumen ecosystem.

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