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Research Article

Isolation and Screening of cellulase producing bacteria from *Capsicum annum* fields of Guntur District, Andhra Pradesh, India

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ABSTRACT

Soil is a rich reservoir of diverse microbial communities capable of producing industrially important enzymes. Cellulases, which hydrolyze cellulose into glucose and related oligosaccharides, play a significant role in biofuel production, waste management, and several biotechnological processes. In this study, cellulase-producing bacteria were isolated from agricultural soil collected from mirchi (*Capsicum annum* L.) fields in Guntur District, Andhra Pradesh, India. Two efficient bacterial isolates, designated as NRI-18 and NRI-19, were screened for cellulase production using Carboxymethyl Cellulose (CMC) agar medium. The isolates were further characterized through morphological, biochemical, and antibiotic susceptibility tests. The formation of clear hydrolysis zones on CMC agar plates confirmed cellulase enzyme production by both isolates. The biochemical profile revealed that the isolates were Gram-negative, MR-positive, catalase-negative, indole-negative, and citrate-negative. The isolates also exhibited distinct antibiotic susceptibility patterns. These findings suggest that agricultural soils harbor potent cellulolytic bacteria with potential applications in enzyme-based biotechnological industries.

INTRODUCTION

Cellulose, the most abundant organic polymer on Earth, constitutes the primary structural component of plant cell walls and represents a renewable source of fermentable sugars for

industrial applications. The enzymatic hydrolysis of cellulose into glucose is primarily carried out by cellulase enzymes, which are composed of endoglucanases, exoglucanases, and β -glucosidases (Lynd et al., 2002). These enzymes have wide-ranging applications in biofuel

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production, the paper and textile industries, food processing, and agricultural residue degradation (Singhania et al., 2013).

Among the various sources of cellulase, microbial cellulases are the most advantageous due to their high catalytic efficiency, environmental adaptability, and ease of large-scale production (Immanuel et al., 2006). Bacteria, in particular, offer advantages over fungi in terms of faster growth rates, thermostability, and the ability to function in a broader range of environmental conditions (Kasana et al., 2008). Soil, especially from agricultural fields rich in plant litter and organic residues, is a promising habitat for cellulolytic microorganisms capable of producing extracellular cellulases.

Screening and isolation of novel cellulase-producing bacteria from such environments can help identify potential candidates for industrial enzyme production and environmental biotechnology. The present study was conducted to isolate, screen, and characterize cellulase-producing bacterial strains from mirchi field soils of Peda Kakani, Guntur District, Andhra Pradesh, and to evaluate their biochemical and antibiotic susceptibility profiles to aid in future biotechnological applications.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected aseptically from mirchi fields located in Peda Kakani, Guntur District, Andhra Pradesh, India. Samples were taken from a depth of 5–10 cm, placed in sterile polyethylene bags, and transported to the laboratory under low-temperature conditions (4 °C) to preserve microbial integrity. The soil was air-dried and subjected to mild heat treatment to

eliminate unwanted moisture and enrich for spore-forming bacteria.

Isolation of Bacterial Strains

Bacterial isolates were obtained using Nutrient Agar Medium (NAM) and Soya Bean Casein Digest Agar (SCDA). Serial dilution of soil samples was carried out up to 10^{-6} and aliquots from each dilution were plated using pour plate and spread plate techniques. The inoculated plates were incubated at 28 ± 2 °C for 24–48 hours. Well-isolated colonies were selected based on morphological distinctness and sub-cultured onto fresh NAM plates to obtain pure isolates. Two predominant isolates were designated as NRI-18 and NRI-19 and stored on nutrient agar slants at 4 °C for further characterization (Prashanthi et al., 2021).

Screening for Cellulase Production

Primary screening for cellulase activity was performed using Carboxymethyl Cellulose (CMC) agar medium containing 1% CMC as the substrate. The isolates NRI-18 and NRI-19 were spot-inoculated on CMC agar plates and incubated at 30 ± 2 °C for 2–4 days. After incubation, the plates were flooded with 1% Congo red solution for 15 minutes, followed by destaining with 1 M NaCl for 10 minutes. The formation of clear halo zones around the colonies indicated cellulose hydrolysis and positive cellulase enzyme activity (Kasana et al., 2008).

Biochemical Characterization

Catalase Test

Isolates were inoculated into Nutrient Broth and incubated for 24–48 hours at 37 °C. Following incubation, 3% hydrogen peroxide (H_2O_2) was added dropwise to each culture tube. The absence



of bubble formation indicated catalase-negative results for both isolates (Chandra, 2023).

Methyl Red–Voges Proskauer (MR–VP) Test

The isolates were grown in MR–VP broth containing buffered peptone (7.0 g/L), dextrose (5.0 g/L), and dipotassium phosphate (5.0 g/L). After 48 hours of incubation, 5–6 drops of methyl red reagent were added. Development of a brick-red coloration in the broth confirmed positive MR activity, while VP reaction was not observed (Sarker et al., 2015).

Indole Test

For indole production, isolates were cultured in tryptophan broth for 24–48 hours at 37 °C. After incubation, 1 mL of xylene was added, followed by 0.5 mL of Kovac's reagent. The absence of a red or pink ring at the surface indicated negative indole production (Alves et al., 2006).

Citrate Utilization Test

Simmon's citrate agar slants were prepared and inoculated with bacterial isolates. The slants were incubated for 24–48 hours at 37 °C. The absence of color change from green to blue and lack of growth indicated negative citrate utilization for both isolates (Iwade et al., 2006).

Microscopic Characterization

Gram staining revealed the morphological characteristics and Gram reaction of the isolates. Both NRI-18 and NRI-19 were found to be Gram-negative rods, appearing pink/red under microscopic examination due to a thinner peptidoglycan layer. For ultrastructural analysis, the isolates were cultured on nutrient agar, fixed, and prepared for Scanning Electron Microscopy (SEM). Additionally, molecular identification was

conducted through PCR amplification and 16S rRNA gene sequencing to confirm bacterial taxonomy (Moyes et al., 2009).

Antibiotic Susceptibility Test

Antibiotic resistance and sensitivity were evaluated using the disc diffusion method on Nutrient Agar plates. Lawn cultures of NRI-18 and NRI-19 were prepared, and antibiotic discs containing ampicillin (AMP 10 µg), chloramphenicol (C 30 µg), novobiocin (NV 5 µg), erythromycin (E 15 µg), and co-trimoxazole (COT 25 µg) were placed aseptically. The plates were incubated at 30 ± 2 °C for 24–48 hours, and the zones of inhibition were measured in millimetres to determine susceptibility patterns (Ataee et al., 2012).

RESULTS

Isolation of Bacterial Strains

From serial dilutions ranging from 10^{-1} to 10^{-6} , multiple distinct bacterial colonies were obtained (Fig. 1). Based on colony morphology and enzyme screening, two isolates exhibiting significant growth and enzyme activity were selected and designated as NRI-18 and NRI-19.



Fig. 1 Isolation of Bacteria

Screening for Cellulase Activity



Both isolates produced clear hydrolysis zones on CMC agar plates after flooding with Congo red solution, confirming cellulase enzyme production (Fig. 2). The medium exhibited an orange discoloration, and transparent halo zones were clearly visible around colonies, corresponding to regions where cellulose had been hydrolyzed by cellulase activity. This indicates that both NRI-18 and NRI-19 are capable of secreting extracellular cellulase enzymes.

Biochemical Tests: All the biochemical characters were represented in table 1.

- **Catalase Test:** No effervescence was observed, confirming both isolates as catalase negative.
- **Methyl Red Test:** Both isolates showed brick-red coloration, indicating positive MR reaction and acid production from glucose fermentation (Fig. 3).
- **Indole Test:** No pink or red color formation occurred, confirming indole-negative results.
- **Citrate Utilization Test:** change in color (medium remained green) was observed confirming citrate-positive reactions (Fig. 3).

Gram Staining

Both NRI-18 and NRI-19 appeared as Gram-negative bacilli, staining pink/red under the microscope, which indicates the presence of a thin peptidoglycan layer and an outer membrane typical of Gram-negative bacteria.

Table 1: Morphological and Biochemical characteristics of NRI-18 and NRI-19

Character	Response of NRI-18	Response of NRI-19
Gram's reaction	+	+
Catalase production	-	-
Methyl red test	-	-
Voges proskauer test	+	+
Indole production	-	-
Citrate utilization	+	+
H ₂ S test	-	-

+: Positive, -: Negative

Antibiotic Susceptibility

Both isolates demonstrated measurable zones of inhibition against selected antibiotics, indicating varying levels of resistance and susceptibility (Fig. 4). The recorded zone diameters confirmed the differential sensitivity of isolates, suggesting possible environmental adaptation and intrinsic resistance mechanisms.



Fig. 2 Cellulase screening activity of the strains NRI-18 and NRI-19



Fig.3 Methyl red test and Citrate utilisation test of the strain NRI-18



Fig. 4 Antibiotic sensitivity of the strain NRI-19

CONCLUSION

The present study successfully isolated and characterized two cellulase-producing bacterial strains (NRI-18 and NRI-19) from mirchi field soils. Both isolates exhibited strong cellulolytic activity on CMC agar, indicating their potential as promising candidates for cellulase production. Their biochemical profiles revealed Gram-positive nature, citrate utilisation tests positive and positive MR reactions, while molecular identification through 16S rRNA sequencing is expected to confirm their taxonomic position. These findings suggest that agricultural soils represent a valuable source of novel cellulase-producing microorganisms with potential industrial and environmental applications.

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Conflict of interest

The authors declare that there is no conflict of interest.

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