



Isolation and Biochemical Characterization of a Robust Cellulase from Haloalkaliphilic *Bacillus* sp. E2 of Lonar Crater Lake

Vishal Dhundale*, Swati Chandak, Rahul Shelke, Jitendra Bajare, Akshata Deshmukh, Shraddha Palekar, Alisha Sayyad, Rutuja Badgude, Vaishnavi Jadhav

Department of Microbiology, Shivaji Mahavidyalaya, Barshi, Maharashtra, India

*Corresponding author: vrdivishal@gmail.com

Received: 10-11-2025; Accepted: 05-12-2025; Published: 31-12-2025

© Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License

<https://doi.org/10.55218/JASR.2025161202>

ABSTRACT

A haloalkaliphilic bacterial strain, designated E2, was isolated from the hypersaline and hyperalkaline Lonar Crater Lake and evaluated for its cellulase production potential. Among seven isolates screened on carboxymethyl cellulose (CMC) agar, E2 exhibited the largest hydrolysis zone, indicating superior cellulolytic activity. Morphological characterization suggested its affiliation with the genus *Bacillus*, which was further supported by its ability to thrive under alkaline and saline conditions. Enzyme activity was optimized with respect to pH, temperature, NaCl concentration, nitrogen source, organic solvents, and metal ions. The cellulase displayed broad pH tolerance (7–12) with an optimum at pH 11, and maintained high activity over a wide temperature range (40–100°C), peaking at 70°C. Maximum enzyme yield was obtained at 3% NaCl, with substantial activity retained up to 7% NaCl, indicating moderate halotolerance. Among nitrogen sources, yeast extract and tryptone supported the highest enzyme production, while ammonium chloride was the least effective. The enzyme showed high stability in the presence of methanol, hexane, and chloroform, but reduced activity with acetone and formaldehyde. Mn^{2+} and Mg^{2+} significantly enhanced cellulase activity, whereas Cu^{2+} caused strong inhibition. Kinetic analysis yielded a K_m of 0.46% CMC and a V_{max} of 178.6 U/mL from Lineweaver–Burk plots, suggesting high catalytic efficiency and good substrate affinity. These results indicate that strain *Bacillus* Strain E2 produces a thermostable, alkali-tolerant, and salt-tolerant cellulase with potential applications in industrial processes such as textile desizing, detergent formulation, pulp biobleaching, and biomass hydrolysis under extreme conditions.

Keywords: Haloalkaliphilic *Bacillus*, Thermostable alkali-tolerant cellulase, Industrial enzyme applications

INTRODUCTION

Cellulose, the most abundant biopolymer on earth, serves as a key structural element of plant cell walls and constitutes a significant portion of agricultural and industrial residues. Structurally, it is a linear polysaccharide consisting of β -1,4-linked glucose units arranged in a crystalline lattice, making it highly resistant to biodegradation. These enzymes convert insoluble cellulose into fermentable sugars, which can be further utilized for the production of bioethanol and other high-value bioproducts.

Microbial cellulases, particularly those from bacterial sources, have attracted considerable interest due to their high catalytic activity, stability under extreme environmental conditions, and cost-effective production using low-priced substrates. While filamentous fungi such as *Trichoderma reesei* and *Aspergillus niger* have long dominated industrial cellulase production, bacterial cellulases are increasingly valued for their robustness and ability to operate in harsh settings, including elevated temperatures, extreme pH, and high salinity (2). Notable cellulase-producing bacteria include members of the genera *Bacillus*, *Cellulomonas*, *Pseudomonas*, and *Thermomonospora*.

Extreme habitats such as soda lakes, hot springs, and salt flats host specialized microbial communities with unique enzymatic capabilities. Lonar Lake, located in Buldhana district, Maharashtra, India (19°58'N, 76°31'E), is an exceptional example of an ancient (approximately 52,000 years old), hyperalkaline (pH 9.5–11.2) and hypersaline (total dissolved solids ~10,000–15,000 mg/L) meteorite impact crater lake. Its unusual combination of high pH, elevated salinity, and mineral-rich composition supports diverse extremophiles, including haloalkaliphilic and alkalitolerant bacteria capable of producing industrially relevant enzymes such as proteases, amylases, and lipases. Despite this, the cellulolytic potential of its microbial community remains largely unexplored.

Investigating such environments provides twofold benefits: the potential discovery of cellulases with novel biochemical properties, and the isolation of extremophilic strains capable of functioning under industrial conditions where conventional enzymes lose activity. With the rising demand for renewable energy and eco-friendly bioconversion processes, the search for efficient cellulase producers from extreme habitats is both timely and necessary.

This study focuses on isolating cellulolytic bacteria from Lonar Lake sediment and water, screening them for cellulase production through qualitative and quantitative assays, and optimizing culture parameters to enhance enzyme yield. Insights gained from this work could advance enzyme biotechnology while enriching our understanding of microbial diversity in extreme ecosystems.

MATERIALS AND METHODS

Sample Collection and Preparation

This study utilized both previously obtained alkaliphilic and halophilic bacterial strains as well as freshly collected soil samples from the Lonar Lake region. Lonar Lake, situated in Buldhana district, Maharashtra, is renowned for its distinct physicochemical characteristics, particularly high alkalinity and salinity, which create a natural niche for extremophiles adapted to thrive under harsh environmental conditions. Soil samples were collected from peripheral zones and shallow regions of the lake using sterile spatulas and transferred into sterile, properly labeled containers. Samples were immediately transported to the laboratory in an insulated icebox and stored at 4 °C until further processing. These pretreated samples served as the source material for cellulase-producing bacterial isolation [1–3].

Enrichment and Isolation of Bacteria on CMC Medium

Cellulase-producing bacteria were enriched using Carboxymethyl Cellulose (CMC) as the sole carbon source. Ten grams of sediment were suspended in 90 mL of sterile distilled water. After allowing the suspension to settle for 10 minutes, 1-mL of the supernatant was inoculated into 100 mL of sterile enrichment broth containing (per liter): 5.0 g CMC, 0.5 g yeast extract, 0.5 g (NH₄)₂SO₄, 0.5 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, and 15.0 g NaCl, with the pH adjusted to 9.5 to promote alkaliphilic and halophilic bacterial growth. Enrichment cultures were incubated at 37°C for 3–5 days under shaking conditions (150 rpm) [4, 5]. Following incubation, serial dilutions up to 10⁻⁷ were prepared in sterile saline (0.85% NaCl), and 100 µL aliquots from each dilution were spread onto CMC agar plates composed of the same basal medium with 1.5% agar. Plates were incubated at 37°C for 48–72 hours. Colonies showing distinct morphological differences were selected and purified by repeated streaking on fresh CMC agar plates. Pure cultures were maintained on CMC slants at 4°C for short-term use and preserved in 20% glycerol stocks at –20°C for long-term storage [6].

Screening of Cellulase-Producing Bacteria by Spot Inoculation

Purified isolates were screened for cellulase activity by spot inoculation onto CMC agar plates. The screening medium consisted of the basal salt medium supplemented with 1% (w/v) CMC and 1.5% (w/v) agar, with the pH adjusted to 9.5 prior to sterilization at 121°C for 15 minutes. Overnight cultures of each isolate were spot-inoculated onto the agar surface using a sterile inoculating loop and incubated at 37°C for 48–72 hours under aerobic conditions. Post-incubation, plates were flooded with 0.1% (w/v) Congo red solution and allowed to stand for 15 minutes at room temperature. Excess

dye was decanted, and plates were rinsed with 1 M NaCl to remove unbound stain. The presence of distinct, clear halos surrounding bacterial colonies indicated CMC hydrolysis, confirming cellulase production.[7]

Enzyme Production and Optimization

Isolates exhibiting strong hydrolysis zones on CMC agar were selected for submerged fermentation to assess cellulase production. A loopful of actively growing culture was inoculated into 100 mL of production medium containing (per liter): 10.0 g CMC, 1.0 g (NH₄)₂SO₄, 1.0 g KH₂PO₄, 0.2 g MgSO₄·7H₂O, and 20.0 g NaCl, with pH adjusted to 9.5. Cultures were incubated at 37 °C on a rotary shaker at 150 rpm for 72 hours. After fermentation, the broth was centrifuged at 10,000 rpm for 15 minutes at 4°C, and the resulting supernatant was collected as crude enzyme extract for further biochemical assays [8, 9].

Protein Estimation Using the Lowry Method

Protein concentration in the crude cellulase extract was determined following the Lowry method using bovine serum albumin (BSA) as the standard protein [10]. A 1 mg/mL stock solution of BSA was prepared and diluted to working concentrations of 20–200 µg/mL. Each tube received 5.0 mL of freshly prepared alkaline copper sulfate reagent (2% Na₂CO₃ in 0.1 N NaOH mixed with 1% CuSO₄·5H₂O and 2% sodium potassium tartrate in a 50:1:1 ratio) and was incubated for 10 minutes at room temperature. This was followed by the addition of 0.5 mL diluted Folin–Ciocalteu phenol reagent and incubation in the dark for 30 minutes. Absorbance was measured at 660 nm, and a standard curve was constructed to calculate the protein content of enzyme samples by interpolation.

Effect of Temperature on Cellulase Activity

The effect of temperature on cellulase activity was determined by assaying enzyme activity at various temperatures while maintaining constant pH and substrate concentration. The assay was performed using 1% (w/v) CMC prepared in 50 mM citrate buffer (pH 5.0). Reaction mixtures containing pre-equilibrated substrate and enzyme were incubated at different temperatures ranging from 20 to 80°C for 10 minutes. After incubation, the amount of reducing sugar released was estimated by the DNS method, and absorbance was recorded at 540 nm using a spectrophotometer. A glucose standard curve was used to calculate enzyme activity, expressed as µmol glucose released per minute per mL of enzyme (U/mL). The relative activity (%) at each temperature was calculated by taking the highest enzyme activity as 100%, and the data were plotted to determine the optimum temperature for cellulase activity. A decrease in enzyme activity at higher temperatures indicated thermal denaturation of the enzyme protein [8, 11].

Effect of pH on Cellulase Activity

The effect of pH on cellulase activity was evaluated by assaying enzyme activity over a pH range of 7.0–12.0, using 1% (w/v) CMC as the substrate and the DNS method to quantify the released reducing sugars. Buffers of equal ionic strength (50 mM) were employed to maintain the desired pH conditions: phosphate buffer (pH 6.0–8.0), Tris–HCl buffer (pH 8.0–9.0), and glycine–NaOH buffer (pH 9.0–10.5). The reaction mixture consisted of 1.0 mL of 1% CMC

solution and 0.1 mL of the crude enzyme extract. The mixture was incubated at 50°C for 10 minutes, after which the reaction was terminated by adding 1.0 mL of DNS reagent. The tubes were then boiled for 5 minutes, cooled to room temperature, and the absorbance was measured at 540 nm using a spectrophotometer. A standard curve was generated using D-glucose to calculate the amount of reducing sugar released. Relative activity (%) was determined, and the enzyme's optimum pH was identified. For pH stability, the enzyme was pre-incubated in respective buffers for 1-hour at 50°C, followed by residual activity measurement under standard assay conditions [12,13].

Effect of NaCl on Cellulase Production

The influence of NaCl concentration on cellulase production was examined by supplementing the production medium with varying NaCl levels (0–7% w/v) prior to sterilization. Cultures were incubated at 30–37°C for 48–72 hours. After centrifugation, the supernatants were assayed for cellulase activity using the DNS method at 540 nm. The activity was expressed as U/mL, and relative activity was compared to the control (0% NaCl) [8,10,14].

Effect of Organic Solvents on Cellulase Activity

To assess solvent stability, the crude enzyme was incubated with 10% (v/v) of various organic solvents, including formaldehyde, methanol, ethanol, isopropanol, acetone, chloroform, hexane, and glycerol, in the presence of 1% CMC at 50°C. Enzyme activity was determined using the DNS method [10]. Solvent tolerance is an important property for potential applications in non-aqueous biocatalysis, as reported for other solvent-stable enzymes [15].

Effect of Metal Ions on Cellulase Activity

The effect of various metal ions was evaluated by adding chloride salts of Na⁺, K⁺, Ca²⁺, Mg²⁺, Mn²⁺, and Cu²⁺ at a final concentration of 5 mM to the standard assay mixture. While certain ions enhanced activity, Cu²⁺ was found to be inhibitory, aligning with reports that metal ions can either stabilize enzyme conformation or disrupt catalytic residues [11,16].

Effect of Nitrogen Sources on Cellulase Production

The influence of different nitrogen sources was tested by supplementing the production medium with 0.5% (w/v) of yeast extract, peptone, soybean meal, ammonium chloride, tryptone, or beef extract, while keeping other components constant. Variations in enzyme yield reflected differential nitrogen utilization, as also reported in studies on bacterial and fungal cellulases [9,16].

RESULTS AND DISCUSSION

Isolation and Characterization of *Bacillus Strain E2*

A total of seven bacterial strains were isolated from sediment and water samples collected from Lonar Lake, a distinctive hypersaline and hyperalkaline ecosystem. These isolates were screened for cellulase production using carboxymethyl cellulose (CMC) agar plates. Among them, the strain designated as *Bacillus Strain E2* exhibited the largest and most distinct hydrolysis zone, indicating

strong cellulolytic potential. Owing to its superior enzyme production capability, *Bacillus Strain E2* was selected for subsequent studies involving cellulase production, optimization, and detailed characterization. This included comprehensive physiological, biochemical, and kinetic analyses to assess its suitability for industrial applications. The colony morphology of *Bacillus Strain E2* was examined on CMC agar plates. Colonies were small (~0.5 mm in diameter), circular, flat in elevation, and white in color, with a moist consistency and an entire margin. The colonies were opaque, indicating dense bacterial growth. These traits are characteristic of smooth, non-pigmented colonies typically observed in environmental *Bacillus* and other aerobic, spore-forming bacteria. Preliminary morphological and cultural observations suggest that *Bacillus Strain E2* belongs to the genus *Bacillus*. The colony characteristics—circular shape, white color, flat elevation, moist texture, entire margins, and opacity—are consistent with those of many *Bacillus* species. Additionally, the small colony size and consistent appearance on nutrient agar further support this preliminary identification. *Bacillus* species are well recognized for their ability to produce a diverse array of industrially important enzymes, including cellulases, and are frequently isolated from alkaline and saline environments such as Lonar Lake. Nonetheless, definitive identification of E2 will require further confirmation through Gram staining, biochemical profiling, and molecular techniques such as 16S rRNA gene sequencing [17–19].

Effect of pH on Cellulase Activity of *Bacillus Strain E2*

The graph illustrates the effect of pH on cellulase activity for *Bacillus Strain E2*, isolated from the hypersaline and hyperalkaline Lonar Crater. Two parameters are plotted: Relative activity (%) and enzyme activity (U/mL) on the Y-axis, across a pH range of 7 to 12. At neutral pH (7), cellulase activity is lowest, with relative activity around 67% and enzyme activity near 85 U/mL. Activity gradually increases with alkalinity, reaching approximately 74% relative activity and 90 U/mL at pH 9. The enzyme demonstrates optimal performance at pH 11, showing maximum relative activity (~81%) and peak enzyme activity (~99 U/mL). At pH 12, both relative and absolute activities remain high, indicating broad alkaline tolerance with negligible decline from the optimum. This alkaline activity profile is consistent with cellulases from haloalkaliphilic *Bacillus* strains adapted to soda-lake environments, where high pH stability is advantageous [20, 2]. Similar trends have been reported for cellulases from *Bacillus halodurans* and *Bacillus alcalophilus*, which exhibit maximal activity in the pH 9–11 range and retain stability

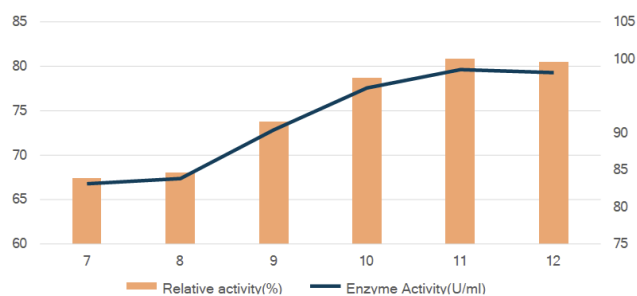


Fig 1: Effect of pH on Cellulase

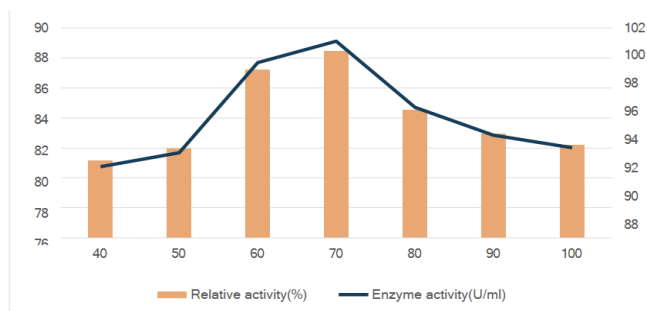


Fig 2: Effect Temperature on Cellulase

under strongly alkaline conditions Fig. 1.[21, 4] The pH adaptability of E2 cellulase suggests potential applicability in industrial processes requiring enzyme function under alkaline conditions, such as textile desizing, detergent formulation, and paper pulp biobleaching

Effect of Temperature on Enzyme Activity

The graph depicts the effect of temperature on cellulase activity for strain E2, isolated from the hypersaline and hyperalkaline Lonar Crater environment. Both Relative Activity (%) and Enzyme Activity (U/mL) are plotted over a temperature range of 40–100°C. At lower temperatures (40–50°C), cellulase activity remains moderate, with relative activity between approximately 81–82% and enzyme activity around 91–92 U/mL. Activity increases sharply between 50 and 60°C, reaching about 87% relative activity and 100 U/mL enzyme activity. The enzyme exhibits its maximum activity at 70°C, with ~89% relative activity and peak enzyme activity of 101 U/mL, suggesting that *Bacillus* strain E2 cellulase is thermostable and adapted to elevated temperatures. Beyond 70°C, enzyme activity gradually decreases, with relative activity dropping to ~85% at 80°C and ~82% at 100°C, indicating partial thermal inactivation at extreme temperatures. Nonetheless, the enzyme retains substantial activity (>90 U/mL) even at 100°C (Fig. 2), demonstrating remarkable thermal tolerance. This thermostability aligns with earlier reports on cellulases from thermophilic and haloalkaliphilic *Bacillus* species isolated from extreme environments, which often maintain catalytic efficiency at elevated temperatures [21,4]. Such heat-tolerant enzymes are advantageous for industrial processes like biomass hydrolysis, textile processing, and pulp biobleaching, where reactions occur under high-temperature conditions to enhance reaction rates and substrate solubility [11,8].

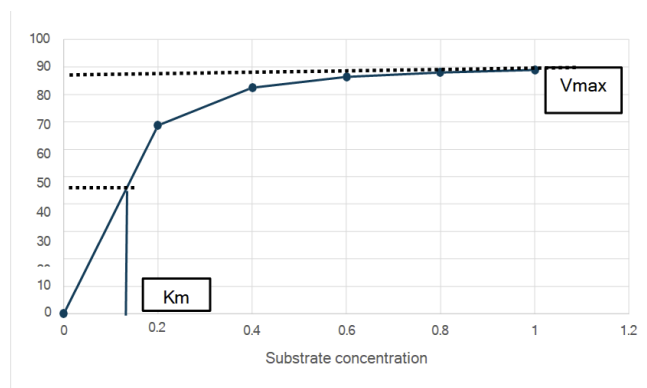


Fig 3: Michaelis-Menten of Cellulase

Michaelis–Menten Kinetics of Cellulase from *Bacillus* Strain E2

The Michaelis–Menten plot for cellulase from *Bacillus* strain E2 illustrates the relationship between substrate concentration and enzyme activity, allowing determination of kinetic parameters V_{max} and K_m . Enzyme activity increases sharply at low substrate concentrations, reaching approximately 70 U/mL at 0.2% CMC (Fig 3). Beyond this point, the rate of increase slows, approaching a plateau at higher substrate concentrations. The V_{max} (maximum velocity) is approximately 88–90 U/mL, representing the catalytic capacity of the enzyme when substrate saturation is achieved. The K_m (Michaelis constant), estimated from the substrate concentration at half of V_{max} (~0.1% CMC), reflects the enzyme's affinity for the substrate—lower K_m values denote higher affinity. The kinetic profile of *Bacillus* strain E2 cellulase, with a relatively low K_m and high V_{max} , indicates strong substrate binding and high catalytic efficiency. Such properties are desirable for industrial applications requiring rapid cellulose hydrolysis, particularly under alkaline conditions. Comparable kinetic patterns have been reported in cellulases from haloalkaliphilic *Bacillus* species and other extremophiles adapted to soda-lake environments [21,4,11].

The Lineweaver–Burk plot for cellulase from E2 provides a linear transformation of the Michaelis–Menten data, allowing more accurate estimation of kinetic constants. In this double reciprocal plot, $1/V$ (enzyme activity⁻¹) is plotted against $1/[S]$ (substrate concentration⁻¹). Based on the Lineweaver–Burk analysis, the intercept on the y-axis ($1/V_{max}$) was 0.0056, corresponding to a V_{max} of approximately 178.57 U/mL as shown in Fig 4. The slope (K_m/V_{max}) was 0.0026, yielding a K_m of 0.464% CMC. These results show that the enzyme exhibits moderate substrate affinity and achieves maximum catalytic efficiency at substrate concentrations near 0.5% CMC. This kinetic behavior is consistent with cellulases from haloalkaliphilic *Bacillus* spp., which are adapted for efficient cellulose degradation in high-pH, saline environments [21,4]. The moderate K_m suggests that the enzyme can function effectively at low to moderate substrate concentrations, an advantage for industrial processes such as textile desizing, pulp biobleaching, and bioethanol production, where substrate levels fluctuate. The high V_{max} reflects strong catalytic turnover once the enzyme–substrate complex is formed, enhancing overall process efficiency

Effect of NaCl on Enzyme Activity

The graph illustrates the effect of NaCl concentration on cellulase production by *Bacillus* strain E2, isolated from the hypersaline and

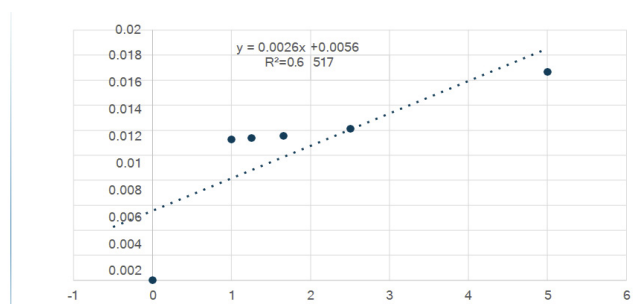


Fig 4: Lineweaver-Burk Plot of Cellulase

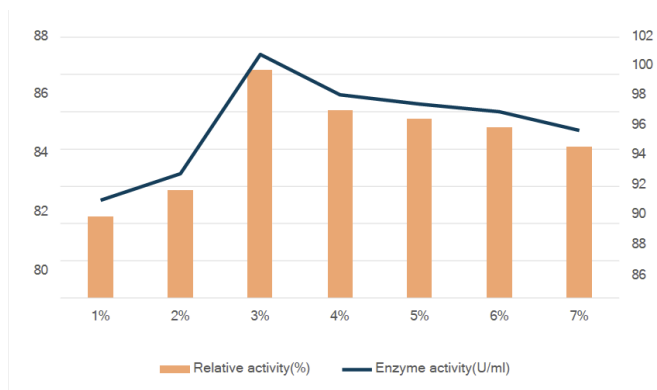


Fig 5: Effect of NaCl on Cellulase

hyperalkaline Lonar Crater. NaCl concentrations ranging from 1% to 7% (w/v) were tested, with Relative Activity (%) and Enzyme Activity (U/mL) plotted on the Y-axes. At lower salinity levels (1–2% NaCl), cellulase production remained moderate, with relative activity between 83–84% and enzyme activity around 94–95 U/mL. Activity peaked at 3% NaCl, reaching ~87% relative activity and ~101 U/mL, suggesting that moderate salinity optimally supports enzyme biosynthesis. Beyond this point, both relative and absolute activities gradually declined, with 84–85% relative activity and 96–97 U/mL observed at 7% NaCl (Fig. 5). This pattern indicates that strain E2 is moderately halotolerant, with an optimum in the low-to-moderate salt range, and retains substantial activity even at higher salinities. Such salt tolerance is consistent with cellulase-producing haloalkaliphilic *Bacillus* species from soda lakes, where moderate salinity enhances enzyme secretion but excessive salinity exerts osmotic stress [4,17]. The ability of E2 to maintain high activity in saline conditions suggests potential industrial applications in textile washing with seawater, saline biomass hydrolysis, and leather processing [11, 22].

Effect of Nitrogen on Enzyme Activity

The graph shows the influence of various nitrogen sources on cellulase production by *Bacillus* strain E2, isolated from the hypersaline and hyperalkaline Lonar Crater ecosystem. The X-axis lists the nitrogen sources (yeast extract, beef extract, soybean meal, ammonium chloride, peptone, and tryptone), while the Y-axes display Relative Activity (%) and Enzyme Activity (U/mL). Yeast extract supported the highest cellulase yield, with relative activity close to 79% and enzyme activity around 99 U/mL. Tryptone also promoted high production (~78% relative activity; ~98 U/mL), followed by peptone

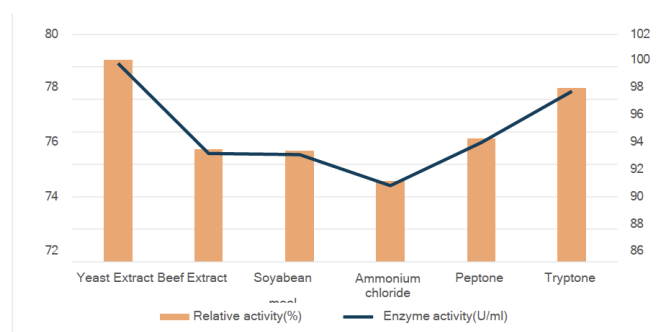


Fig 6: Effect of nitrogen source

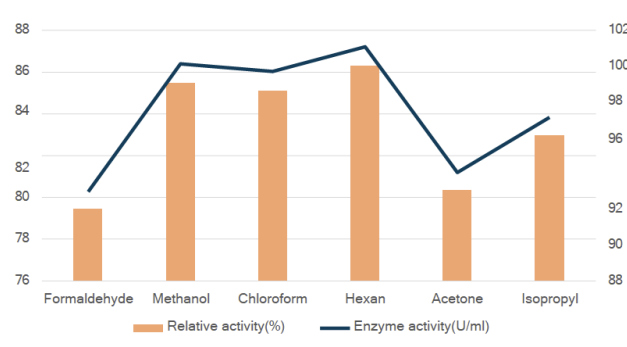


Fig 7: Effect of solvent

(~77%; ~96 U/mL). Moderate yields occurred with beef extract and soybean meal (~76%; ~95 U/mL). The lowest activity was observed with ammonium chloride (~75%; ~93 U/mL) (Fig. 6), indicating that E2 performs better with complex organic nitrogen sources than with inorganic nitrogen. This preference agrees with reports that organic nitrogen sources (e.g., yeast extract, tryptone) enhance bacterial cellulase production by providing amino acids, vitamins, and cofactors that stimulate enzyme biosynthesis [9, 16]. In contrast, inorganic nitrogen sources like ammonium salts may limit yields due to their restricted nutrient composition. Similar findings have been documented for cellulase-producing *Bacillus* spp. from alkaline and saline environments [4, 21].

Effect of Solvent on Enzyme Activity

The graph shows the effect of different organic solvents (10% v/v) on the activity of cellulase produced by *Bacillus* strain E2. Relative activity (%) and enzyme activity (U/mL) were measured after exposure to each solvent. Among the tested solvents, hexane yielded the highest enzyme activity (~87% relative; ~101 U/mL), followed by methanol (~86%; ~100 U/mL) and chloroform (~85%; ~100 U/mL). Isopropanol supported moderate stability (~83%; ~97 U/mL), whereas acetone (~81%; ~95 U/mL) and especially formaldehyde (~79%; ~94 U/mL) caused greater inhibition (Fig. 7). The ability of E2 cellulase to retain high activity in the presence of certain organic solvents—particularly non-polar ones like hexane—suggests potential for non-aqueous biocatalysis, where organic solvents enhance substrate solubility or shift reaction equilibria [15]. Solvent stability is a valuable trait for industrial enzymes used in biodiesel synthesis, fine-chemical production, and biotransformations [23]. However, polar solvents and reactive aldehydes may disrupt protein conformation, reducing activity.

Effect of Metal Ions on Enzyme Activity

The graph illustrates the influence of various metal ions (5 mM) on cellulase activity produced by *Bacillus* strain E2. Among the tested ions, Mn^{2+} showed the most pronounced stimulatory effect, producing the highest activity (~106% relative; ~102 U/mL). Mg^{2+} also enhanced cellulase activity (~104%; ~101 U/mL). Moderate improvements occurred with Na^+ and Ca^{2+} (both ~98%; ~99–100 U/mL). K^+ had a neutral to slightly inhibitory effect (~96%; ~98 U/mL), while Cu^{2+} caused a marked reduction (~88%; ~90 U/mL), indicating strong inhibition (Fig. 8). The stimulatory roles of Mn^{2+} and Mg^{2+} align with previous reports showing that certain divalent cations stabilize cellulase conformation and promote catalytic

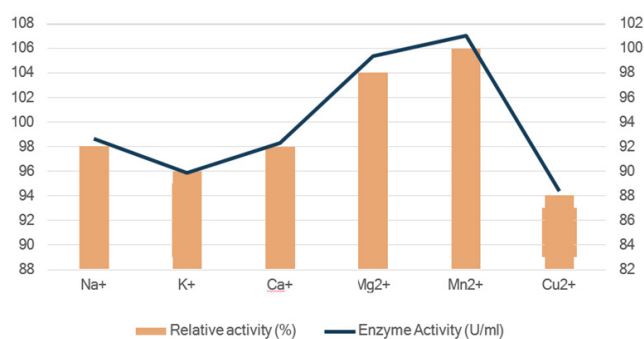


Fig 8: Effect of Metal Ion on cellulase

efficiency [11,16]. Conversely, Cu^{2+} inhibition is well-documented and attributed to its strong affinity for thiol groups in proteins, causing conformational disruption [9]. These results suggest that E2 cellulase is a metal-ion-responsive enzyme, exhibiting optimal activity in the presence of Mn^{2+} and Mg^{2+} , making it suitable for biotechnological processes involving such ions.

CONCLUSION

The present study demonstrates that *Bacillus* strain E2, isolated from the hypersaline and hyperalkaline Lonar Crater Lake, is a potent producer of a thermostable, alkali-tolerant, and moderately halotolerant cellulase. The enzyme exhibited optimal activity at pH 11 and 70°C, with substantial stability over a wide range of pH, temperature, and salinity conditions. Organic nitrogen sources, particularly yeast extract and tryptone, significantly enhanced enzyme production, while ammonium chloride was less effective. The cellulase retained high activity in the presence of certain organic solvents such as methanol, hexane, and chloroform, and was strongly stimulated by Mn^{2+} and Mg^{2+} , but inhibited by Cu^{2+} . Kinetic parameters ($K_m = 0.46\%$ CMC; $V_{max} = 178.6$ U/mL) indicate good substrate affinity and high catalytic efficiency. These combined properties suggest that *Bacillus* strain E2 cellulase is well suited for industrial applications in processes requiring enzyme stability under extreme alkaline, thermal, and saline conditions, such as textile desizing, detergent formulation, paper and pulp biobleaching, and biomass saccharification for biofuel production. Further molecular identification and scale-up studies are warranted to explore its full biotechnological potential.

REFERENCES

- Jones BE, Grant WD, Duckworth AW, & Owenson GG (1998). Microbial diversity of soda lakes. *Extremophiles*, 2(3), 191–200. <https://doi.org/10.1007/s007920050060>
- Wani AA, Sharma D, & Ghosh TK (2016). Microbial diversity of Lonar Lake: A model for exobiology. *Journal of Astrobiology & Outreach*, 4(2), 1–6. <https://doi.org/10.4172/2332-2519.1000143>
- Sangode SJ, Rajan S, Meshram DC, Lakshmanan E, & Patil SG (2020). Sediment and water geochemistry of Lonar Crater Lake, India: Implications for astrobiology and crater lake evolution. *Quaternary International*, 553, 79–96. <https://doi.org/10.1016/j.quaint.2020.02.036>

- Gupta P, Samant, K, & Sahu A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*, 2012, Article 578925. <https://doi.org/10.1155/2012/578925>
- Kasana RC, Salwan R, Dhar H, Dutt S, & Gulati A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Current Microbiology*, 57(5), 503–507. <https://doi.org/10.1007/s00284-008-9276-8>
- Ariffin H, Abdullah N, UmiKalsom MS, Shirai Y, & Hassan MA (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. *International Journal of Engineering and Technology*, 3(1), 47–53.
- Teather RM, & Wood PJ (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and Environmental Microbiology*, 43(4), 777–780. <https://doi.org/10.1128/aem.43.4.777-780.1982>
- Lynd LR, Weimer PJ, van Zyl WH & Pretorius, IS (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3), 506–577. <https://doi.org/10.1128/MMBR.66.3.506-577.2002>
- Sadhu S, & Maiti TK (2013). Cellulase production by bacteria: A review. *British Microbiology Research Journal*, 3(3), 235–258. <https://doi.org/10.9734/BMRJ/2013/2367>
- Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*. 1959;31(3):426-428.
- Bhat M.K. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18(5), 355–383. [https://doi.org/10.1016/S0734-9750\(00\)00041-0](https://doi.org/10.1016/S0734-9750(00)00041-0)
- Ghose TK. Measurement of cellulase activities. *Pure Appl Chem*. 1987;59(2):257-268.
- Mandels M, Weber J. The production of cellulases. *Adv Chem Ser*. 1969;95:391-414.
- Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. (duplicate entry) A rapid method for detection of microbial cellulases on agar plates using Gram's iodine. *Curr Microbiol*. 2008;57:503-507.
- Ogino H, Ishikawa H. Enzymes which are stable in the presence of organic solvents. *J Biosci Bioeng*. 2001;91(2):109-116.
- Immanuel G, Dhanusha R, Prema P, Palavesam A. Effect of different growth parameters on endoglucanase enzyme activity by bacterial isolate *Cellulomonas* sp. *Appl Environ Microbiol*. 2006;5(16):1882-1886.
- Horikoshi K. Alkaliphiles: some applications of their products for biotechnology. *Microbiol Mol Biol Rev*. 1999;63(4):735-750.
- Logan NA, van Vos P. Genus *Bacillus* Cohn 1872. In: De Vos P, et al., eds. *Bergey's Manual of Systematic Bacteriology*. 2nd ed, Vol 3. New York: Springer; 2009. p. 21-128.
- Mandic-Mulec I, Stefanic P, van Elsas JD. Ecology of *Bacillus* and related genera: lessons from environmental genomics. *FEMS Microbiol Ecol*. 2015;91(6):fiv053.
- Horikoshi K. Alkaliphiles: some applications of their enzymes. *Extremophiles*. 1999;3(2):85-90.
- Acharya S, Chaudhary A. Alkaline cellulase produced by a newly isolated *Bacillus* sp. and its application in bio-bleaching of kraft pulp. *J Microbiol Biotechnol Res*. 2012;2(1):155-162.
- Ventosa A, Nieto JJ, Oren A. Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev*. 1998;62(2):504-544.
- Klibanov AM. Improving enzymes by using them in organic solvents. *Nature*. 2001;409:241-246.

HOW TO CITE THIS ARTICLE: Dhundale V, Chandak S, Shelke R, Bajare J, Deshmukh A, Palekar S, Sayyad A, Badgude R, Jadhav V. Isolation and Biochemical Characterization of a Robust Cellulase from Haloalkaliphilic *Bacillus* sp. E2 of Lonar Crater Lake. *J Adv Sci Res*. 2025; 16(12): 7-12 DOI: 10.55218/JASR.2025161202