

# Isolation and Screening of Cellulase Producing Bacterial Strain from Meteoritic Crater of Lonar Lake

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## Abstract

Lonar is the soda lake comprising biotechnologically important microbes with industrially important enzymes. The potential of the extremozymes is still underestimated. Lonar Lake has to be explored for its uniqueness, as it has the unique environmental condition with high salinity i. e. Halophilic condition and the high pH value i. e. alkaliphilic condition, the pH value reaches up to 10.5 and in such unique environment, cellulose is one of the largest organic components. In the present investigation, different samples from soil, water & sediment were collected for the isolation of potential cellulase producing bacteria. Among the collected samples, sediment microflora was isolated on CMC (Carboxy Methyl Cellulose) agar for the identification of cellulase producer. Total five isolates were screened on CMC agar medium among them LLI-1 (Lonar Lake Isolate) isolate showed maximum cellulolytic activity which was indicated by zone of clearance around the bacterial colony. The same bacterium was tentatively identified by culture dependent methods as *Bacillus Sp.* The potent Cellulase producer was enriched in CMC broth having pH 10.5 and incubated at 37°C for 24 hr.

**Key words:** *Bacillus Sp.*, CMC, Lonar lake, Isolates

## 1. Introduction

The Lonar Crater Lake is a Lonar Soda Lake situated in the Buldhana District of Maharashtra State of India. The uniqueness of Lonar Lake is its salinity and alkalinity which harbors various unidentified, unique haloalkaliphilic bacterial species. Lonar is the largest crater among three which is formed due to meteoritic impact 50000 years ago [1]. The genus *Bacillus* comprises a variety of industrially important species and has a

history of safe use in both food and pharmaceutical industry. There is a diversity in the hydrolytic activity of an enzyme in saline and hypersaline environment [2]. Alkaliphilic microorganisms, in particular *Bacillus* species, have attracted much interest because of their ability to produce extracellular metabolites that are active and stable at high pH [3, 4]. Halophilic bacteria from marine environment are also better sources of enzymes of industrial importance that may have potential of pharmaceutical and biotechnological applications [5]. The TDS of Lonar is very high as compared to African soda lake and Kenyan soda lake [6, 7]. In Lonar, no significant studies have been conducted so far to isolate and produce enzymes. Therefore, present study was intended to isolate, screen and characterize cellulase producing bacteria from Lonar Lake which could be further explored for its biotechnological potential.

## 2. METHODOLOGY

### Sample collection:

The sediment sample was collected from Lonar lake in sterile screw cap tubes and used for the isolation of haloalkaliphilic cellulase producing microbes.

### Isolation of Cellulase Producing Bacteria:

The collected sample was diluted and streaked over the Nutrient agar plates and CMC agar plates. All the plates were incubated at 37°C for 24 to 48 hrs. The medium Carboxy Methyl Cellulose (CMC) broth pH 10.5 was used for the enrichment of the cellulolytic bacteria. (composition: Carboxy methyl cellulose 2.0g, sodium nitrate-1.0g, dipotassium Phosphate-1.0 g, Potassium Chloride-1.0g, Magnesium sulphate-0.5g, Ferrous sulphate-0.01, yeast extract-5.0, pH-10.5± 0.2 and incubated at 37°C for 24 hrs).

### Screening and identification of cellulase producing bacteria:

Pure culture of isolate was streaked on CMC agar plate and incubated for 24-48 hrs at 37°C. after incubation, the plates were flooded with 1% Congo red and kept for 15 - 20 min. 1 M NaCl solution was used to treat the

flooded plate. Zone of clearance around the colony indicates the hydrolysis of cellulose.

## 3. RESULTS AND DISCUSSION

Lonar meteoritic crater is one of the unique and largest craters of its kind to find biotechnologically important microbes having potential to produce different kinds of secondary metabolites includes, enzymes, antibiotics, etc.

Haloalkaliphilic microbes are commonly found in the Lonar crater including soil, sediment and water. In the present investigation sediment sample was collected in a sterile Screw cap tube to minimize the environmental contamination of the surroundings. The sediment sample was enriched in CMC broth to obtain the maximum number of cells at 37°C for 24 hrs. After incubation the enriched sample was streaked on CMC agar plates and incubated at 37°C for 24 hrs. After incubation various microbial colonies were observed on plates among them five isolates were designated as Lonar Lake Isolate (LLI) as LLI-1, LLI-2, LLI-3, LLI-4, LLI-5. The biochemical characters of isolates were studied (Table 1).

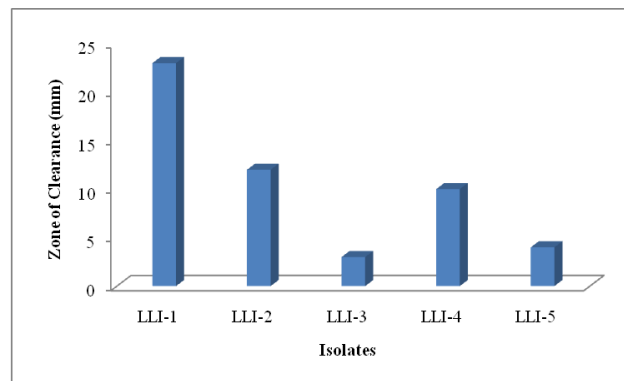
Among five, the three isolates showed potential to produce cellulase and two namely LLI-3 & LLI-5 showed lower cellulolytic activity on CMC agar plates. The LLI-1 is one of the most efficient cellulase producing strain showed 23 mm zone of clearance on CMC agar plates (Fig:1).

According to Siddarthan *et al.* [5] studied on halophilic bacterial strain *Bacillus subtilis* and *Bacillus endophyticus* showed cellulase activity of 14.87 and 16.83 respectively. They also studied cellulolytic activity of Psychrophiles and thermophiles in comparison with Halophilic bacteria.

The LLI-1 was tentatively identified by culture dependent method as *Bacillus Sp.* The potent cellulase producer was enriched in CMC broth pH 10.5 at 37°C for 24 hr. another media i.e. Horikoshi media and

**Table1: The Biochemical characters of isolates.**

Character	LLI-1	LLI-2	LLI-3	LLI-4	LLI-5
Morphology	Rod	Rod	Rod	Cocci	Rod
Gram nature	+	+	+	+	+
Motility	Motile	Motile	Motile	Non-motile	Motile
Oxidase	+	+	+	+	-
Catalase	+	+	+	+	-
pH range	7 to 10.5	7 to 10.5	7 to 10.5	7 to 10.5	7 to 10.5
Urease	-	-	-	-	+
Nitrate reduction	+	+	+	+	-
H <sub>2</sub> S production	-	-	-	-	-
Lipase	+	+	+	+	+
Protease	+	+	+	+	-
Cellulase	+	+	-	+	-
Gelatin	+	+	+	-	+
Starch	+	+	+	+	+
D-Glucose	+	+	+	+	-
Lactose	+	+	+	-	+

**Fig.1: Zone of hydrolysis of cellulase producers**

nutrient broth with pH 10.5 was also used for the enrichment of isolate. Also on these media the isolate shows maximum growth.

In previous investigation, Kanekar *et al.* [1] was isolated number of other bacterial species except *Bacillus sp.* These are *Rhodobacteriaceae sp.*, *Thermoactinomyces thalophilus*, *Matinobacter alkaliphilus etc.* from alkaline lonar lake.

Present investigation concludes that, the haloalkaliphilic bacterial species from Lonar Meteoritic crater showed the significant cellulolytic activity which could be useful in industry.

#### Conflict of interest

No conflict of interest influenced in this research.

#### 4. REFERENCES

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