E-ISSN No : 2456-5040 | Volume : 11 | Issue : 9 | Sep 2026

HYDROLYTIC ENZYME PRODUCING ACTINOMYCETES FROM THE ALKALINE LONAR CRATER ECOSYSTEM AND THEIR BIOTECHNOLOGICAL POTENTIAL

Access Online

Article DOI:
10.5281/zenodo.23014492

G. G. Dhage

Department of Microbiology, Shri Vyankatesh Arts, Commerce and Science College Deulgaon Raja, Buldhana. Maharashtra, India

How to Cite:
G. G. Dhage (2026), Hydrolytic Enzyme Producing Actinomycetes from The Alkaline Lonar Crater Ecosystem and Their Biotechnological Potential, International Educational Applied Scientific & Research Journal (IEASRJ), Vol: 11, Issue: 9, 38-44

ABSTRACT

Actinomycetes are well known for their ability to produce a wide range of extracellular enzymes and therefore represent an important source of industrially useful biocatalysts. In the present study, actinomycetes isolated from the extreme environmental conditions of Lonar Crater, Maharashtra, India, were investigated for their ability to produce hydrolytic enzymes. Isolates recovered from water, sediment, and microbial mat samples were screened qualitatively for four extracellular enzymes, namely protease, amylase, cellulase, and lipase as these are the main hydrolytic enzymes. The isolates showed noticeable differences in their enzyme-producing profiles. The isolates were tentatively identified in the analysis based on morphological, physiological, biochemical and cultural characteristics. *Nocardiopsis synnemataformans* was positive for protease, amylase, and cellulase production but did not exhibit lipase activity. In contrast, *Streptomyces viridodiastaticus* produced protease, amylase, and lipase, while cellulase activity was not detected. Among the tested isolates, *Nocardiopsis valiformis* and *Nocardiopsis alba* showed positive reactions for all four enzymes. These findings indicate that actinomycetes inhabiting the Lonar Crater ecosystem possess varied hydrolytic capabilities, which may be associated with their adaptation to the physicochemical conditions of this unusual environment. The enzyme-positive isolates, particularly those exhibiting multiple activities, could serve as promising candidates for further studies involving enzyme production, optimization of culture conditions, purification, characterization, and assessment of potential industrial applications.

KEYWORDS: Actinomycetes, Lonar Crater, Hydrolytic Enzymes, Protease, Amylase, Cellulase, Lipase

1. INTRODUCTION

Actinomycetes constitute a diverse group of filamentous, Gram-positive bacteria that are widely recognized for their remarkable metabolic versatility. They occur in a variety of habitats, ranging from ordinary soils and aquatic environments to highly saline, alkaline and other stressful ecosystems. Their ability to produce antibiotics, pigments, extracellular enzymes and several other bioactive metabolites has made them an important group for microbiological and biotechnological research. Their broad metabolic potential has consequently encouraged researchers to explore actinomycetes from less-studied habitats as potential sources of useful enzymes and secondary metabolites.

Extracellular hydrolytic enzymes produced by microorganisms have considerable importance because of their ability to break down complex biological polymers into simpler compounds. Among these enzymes, proteases, amylases, cellulases and lipases are particularly relevant to both industrial and environmental processes. Proteases are involved in the degradation of proteins and are widely used in detergent, food, leather and pharmaceutical industries.

Original Research

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Amylases play an important role in the hydrolysis of starch and are commonly utilized in food processing, baking, brewing and starch-processing industries. Cellulases are involved in the breakdown of cellulose and are therefore relevant to lignocellulosic biomass conversion and several environmental applications. Lipases, on the other hand, hydrolyze triglycerides and have applications in food processing, detergents, pharmaceuticals and other biotechnological processes. The ability of microorganisms to produce more than one type of hydrolytic enzyme can therefore increase their value as sources of multifunctional biocatalysts.

Interest in microbial enzymes has gradually extended beyond conventional microorganisms to organisms adapted to extreme environments. Microorganisms living under conditions such as high salinity, elevated temperature, acidic or alkaline pH, and other environmental stresses have developed physiological and biochemical mechanisms that allow them to survive under conditions unsuitable for many ordinary microorganisms. Enzymes produced by such organisms may consequently possess properties that are useful for processes carried out under similarly demanding conditions. In particular, microorganisms adapted to saline and alkaline environments are considered promising sources of enzymes that may retain activity or stability under high salt concentrations or alkaline pH. Thus, extreme environments represent valuable natural reservoirs for the discovery of microorganisms with distinctive metabolic characteristics.

Among the natural alkaline habitats of India, Lonar Crater in Buldhana district of Maharashtra provides an unusual and scientifically valuable environment for microbial exploration. The crater was formed by a meteorite impact in basaltic rock and contains a saline and highly alkaline lake ecosystem. Microbiological investigations have shown that the lake supports a diverse microbial community, with members of Actinobacteria representing an important component of the bacterial population (Kumbhare *et al*., 2015). Earlier culture-based studies also demonstrated the occurrence of alkaliphilic bacteria in Lonar Lake, indicating that microorganisms capable of adapting to its distinctive physicochemical conditions can be recovered from the ecosystem (Joshi *et al*., 2008).

The occurrence of actinomycetes adapted to the Lonar environment has been demonstrated by the isolation and characterization of *Streptomyces lonarensis*, an alkaliphilic actinomycete obtained from soil in the vicinity of Lonar Lake. The organism was reported to grow over a broad pH range of 7–11, providing evidence that Lonar-associated habitats can harbour actinomycetes capable of tolerating alkaline conditions (Sharma *et al*., 2016).

Although Lonar Lake has attracted considerable attention for its microbial diversity and the isolation of metabolically interesting microorganisms, the hydrolytic enzyme-producing potential of its culturable actinomycetes warrants further investigation. Previous research on the Lonar ecosystem has already indicated its potential as a source of microorganisms and metabolites of industrial and pharmaceutical interest (Singh *et al*., 2024). However, comparative screening of actinomycete isolates for multiple hydrolytic activities can provide useful preliminary information for selecting promising strains for subsequent studies.

In the present investigation, the isolated actinomycetes were screened for their ability to produce four extracellular hydrolytic enzymes, namely protease, amylase, cellulase and lipase. The isolates displayed different enzyme-producing profiles.

Accordingly, the objective of the present study was to screen actinomycetes isolated from the extreme Lonar Crater environment for the production of selected extracellular hydrolytic enzymes -protease, amylase, cellulase and lipase and to compare the enzyme-producing profiles of the isolates. The study was intended as an initial screening step for identifying promising actinomycete strains that could be investigated further through quantitative enzyme production, optimization of culture conditions and biochemical characterization.

2. MATERIALS AND METHODS

2.1 Study site and sample collection

The present investigation was carried out using actinomycete isolates obtained from the Lonar Crater ecosystem, located in Buldhana district of Maharashtra, India. The Lonar ecosystem is characterized by its unusual alkaline and saline

conditions, making it a suitable environment for investigating microorganisms adapted to extreme habitats. Samples were collected from different ecological niches within the crater, including water, sediment and microbial mat, to obtain a diverse range of actinomycete isolates.

The collected water samples were alkaline in nature.. Samples from the selected sites were collected aseptically and transported to the laboratory for further microbiological analysis.

2.2 Isolation of actinomycetes

For selective isolation, the collected water, sediment and microbial mat samples were pretreated using heat and 1% $CaCO_3$ treatment. The treated samples were inoculated onto selective media such as SCA, AIA, ISSA, OA, ISP-5 and YEMA, supplemented with griseofulvin to inhibit fungal growth. After incubation, colonies with typical actinomycete characteristics were selected and purified by repeated subculturing. A total of **45 isolates** were obtained, with SCA showing the highest recovery.

2.3 Maintenance and preliminary characterization of isolates

The isolates obtained during the primary screening were purified and maintained on suitable culture media for subsequent experiments. Preliminary identification was based on a combination of cultural, morphological and biochemical characteristics, as these features provide useful initial information for differentiating actinomycete isolates.

2.4 Screening of actinomycetes for hydrolytic enzyme production

The recovered actinomycetes were screened for their ability to produce four important extracellular hydrolytic enzymes: protease, amylase, cellulase and lipase. Qualitative screening was carried out by growing the isolates on media containing the respective substrates. Enzyme production was inferred from the ability of an isolate to degrade the substrate surrounding its colony.

The appearance of a distinct clear or hydrolysis zone around the growing colony was considered a positive indication of extracellular enzyme production. Qualitative plate assays of this type are commonly employed for the preliminary screening of microbial enzyme producers (Hankin and Anagnostakis, 1975; George *et al*., 2001).

2.4.1 Protease screening

Proteolytic activity of the isolates was examined using a protein-containing screening medium. After inoculation and incubation, the plates were observed for degradation of the protein substrate surrounding the colonies. Development of a distinct clear zone around an isolate was recorded as evidence of extracellular protease production.

2.4.2 Amylase screening

Amylase production was evaluated using a starch-containing medium. The isolates were inoculated onto the screening plates and allowed to grow under the conditions used in the study. Following incubation, the plates were examined for evidence of starch degradation surrounding the colonies. A visible hydrolysis zone was considered indicative of amylolytic activity.

2.4.3 Cellulase screening

The cellulolytic potential of the isolates was assessed using a medium containing a cellulose-based substrate. After inoculation and incubation, the plates were examined for substrate degradation around the growing colonies. The formation of a distinct clearance or hydrolysis zone was recorded as a positive cellulase reaction.

2.4.4 Lipase screening

Lipase production was investigated using a lipid-containing screening medium. Following inoculation and incubation, the cultures were examined for visible changes in the substrate surrounding the colonies. The development of a recognizable hydrolysis zone was considered evidence of extracellular lipase activity.

2.5 Selection and identification of enzyme-producing isolates

Based on the results of the preliminary enzyme screening, isolates showing hydrolytic activity were selected for further investigation. Their cultural, morphological and biochemical characteristics were examined in greater detail.

2.6 Evaluation of enzyme-producing profiles

The enzyme-producing characteristics of the selected actinomycete isolates were compiled for protease, amylase, cellulase and lipase. Based on the screening results, isolates were grouped according to their ability to produce individual enzymes or combinations of two or more enzymes.

The resulting enzyme profiles were compared among the identified isolates to determine the extent of their hydrolytic enzyme-producing potential. This comparison provided an overall indication of the diversity of extracellular enzyme activities among actinomycetes recovered from the Lonar Crater ecosystem.

3. RESULTS AND DISCUSSION

3.1 Extracellular enzyme production by actinomycete isolates

Eight selected actinomycete isolates were screened qualitatively for the production of four extracellular hydrolytic enzymes: protease, lipase, amylase and cellulase. Considerable variation was observed in the enzyme-producing profiles of the isolates. Overall, lipase and cellulase activities were the most frequently detected (87.5% each), followed by amylase (75.0%) and protease (62.5%).

Among the tested isolates, *Nocardiopsis valiformis* and *Nocardiopsis alba* showed positive activity for all four enzymes, indicating a broad hydrolytic potential. *Nocardiopsis synnemataformans* exhibited protease, amylase and cellulase activities but was negative for lipase. In contrast, *Streptomyces viridodiastaticus* showed protease, lipase and amylase activity but did not produce detectable cellulase. The remaining isolates displayed different combinations of enzyme activities.

The ability of several isolates to produce multiple extracellular enzymes indicates considerable metabolic versatility among the actinomycetes. Such organisms can secrete hydrolytic enzymes capable of degrading diverse organic substrates and therefore have considerable potential for industrial and environmental applications.

3.2 Protease activity

Protease activity was detected in five isolates (62.5%): *Nocardiopsis valiformis*, *Nocardiopsis synnemataformans*, *Streptomyces viridodiastaticus*, *Nocardiopsis alba* and *Arthrobacter* sp. The remaining isolates did not exhibit detectable proteolytic activity under the screening conditions.

The formation of a clear hydrolysis zone around the growing colonies indicated extracellular degradation of the protein substrate. The protease-producing ability of these isolates suggests their potential for the production of extracellular proteases with possible applications in detergent, food, leather and other biotechnological processes.

The occurrence of protease-producing actinomycetes in the present study is particularly interesting because microorganisms from alkaline environments may produce enzymes capable of functioning under relatively high-pH conditions. Further quantitative and biochemical characterization would be necessary to establish the industrial suitability of these enzymes.

3.3 Lipase activity

Lipase was the most widely detected enzyme together with cellulase, with seven isolates (87.5%) showing positive activity. Lipase production was observed in *Nocardiopsis valiformis*, *Nocardiopsis* sp., *Streptomyces viridodiastaticus*, *Brachybacterium grinsengisoli*, *Nocardiopsis alba*, *Arthrobacter* sp. and *Streptomyces* sp. Only *Nocardiopsis synnemataformans* was negative for lipase activity.

The high frequency of lipase-positive isolates demonstrates the strong lipolytic potential of the selected actinomycetes. Extracellular lipases are important industrial enzymes with applications in food processing, detergents, leather processing, biocatalysis and biodiesel production. The isolates obtained in the present study could therefore serve as potential candidates for further screening and optimization of lipase production.

3.4 Amylase activity

Amylase activity was observed in six isolates (75.0%), namely *Nocardiopsis valiformis*, *Nocardiopsis synnemataformans*, *Nocardiopsis sp.*, *Streptomyces viridodiastaticus*, *Nocardiopsis alba* and *Streptomyces* sp. No detectable amylase activity was observed in *Brachybacterium grinsengisoli* and *Arthrobacter sp.*

The presence of amylolytic activity indicates the ability of these isolates to secrete enzymes capable of hydrolyzing starch. Microbial amylases are commercially important and are extensively used in starch processing, food industries, detergents, textiles and related biotechnological processes. The relatively high occurrence of amylase-producing isolates therefore emphasizes the enzyme-producing potential of the actinomycete collection.

3.5 Cellulase activity

Cellulase activity was detected in seven isolates (87.5%). Positive activity was observed in *Nocardiopsis valiformis*, *Nocardiopsis synnemataformans*, *Nocardiopsis sp.*, *Brachybacterium grinsengisoli*, *Nocardiopsis alba*, *Arthrobacter* sp. and *Streptomyces sp.* Only *Streptomyces viridodiastaticus* showed negative cellulase activity.

The high frequency of cellulase-producing isolates indicates their potential to degrade cellulose and other cellulosic substrates. Cellulolytic actinomycetes are of considerable interest for agricultural waste degradation, biomass conversion, pulp and paper processing and other industrial applications. The present findings therefore suggest that the selected isolates may have potential for further investigation as sources of cellulolytic enzymes.

3.6 Comparative enzyme-producing potential

The overall enzyme profile revealed that most of the selected actinomycetes produced more than one extracellular enzyme. *Nocardiopsis valiformis* and *Nocardiopsis alba* exhibited the broadest enzyme profile, with positive results for protease, lipase, amylase and cellulase. The percentage distribution of enzyme-positive isolates was as given in table no. 1:

Table 1: percentage distribution of enzyme-positive isolates

Enzyme	Positive isolates	Percentage
Protease	5/8	62.5%
Lipase	7/8	87.5%
Amylase	6/8	75.0%
Cellulase	7/8	87.5%

The predominance of lipase and cellulase activities, together with the occurrence of multiple enzyme activities in individual isolates, demonstrates the broad extracellular hydrolytic capability of the selected actinomycetes. These findings support the view that actinomycetes inhabiting the alkaline–saline Lonar ecosystem represent a promising source of enzymes with potential biotechnological importance.

Fig. Enzymatic activity profile of actinomycetes isolates.

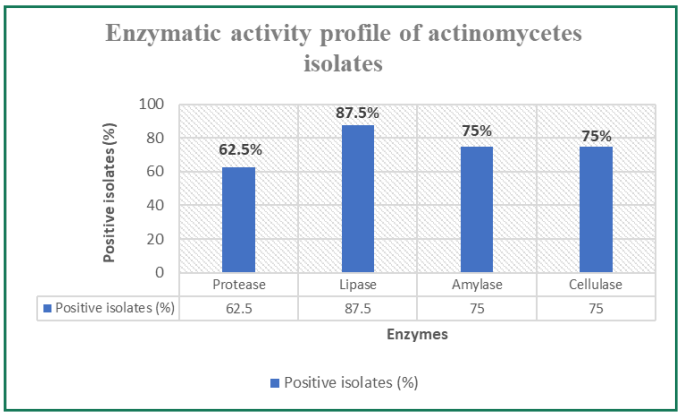

Table 2: Enzymatic activity of Actinomycetes isolates

Sr. No.	Isolate code	Tentative identified Isolate	Protease	Lipase	Amylase	Cellulase
1	GGD02	*Nocardiopsis valiformis*	+	+	+	+
2	GGD08	*Nocardiopsis synnemataformans*	+	-	+	+
3	GGD10	*Nocardiopsis spp.*	-	+	+	+
4	GGD12	*Streptomyces viridodiastaticus*	+	+	+	-
5	GGD24	*Brachybacterium grisengisoli*	-	+	-	+
6	GGD25	*Nocardiopsis alba*	+	+	+	+
7	GGD45	*Arthrobacter spp.*	+	+	-	-
8	GGD47	*Streptomyces spp.*	-	+	+	+

(+ = Enzyme activity show by isolate, - = Enzyme activity did not show by isolate)

Fig. Protease production by isolate GGD02

Fig: Lipase production by isolate GGD02

4. CONCLUSION

The present study demonstrated substantial extracellular enzyme-producing potential among the selected actinomycete isolates. Of the four enzymes investigated, lipase and cellulase showed the highest frequency of occurrence (87.5% each), followed by amylase (75.0%) and protease (62.5%). Most isolates exhibited activity for multiple enzymes, indicating considerable metabolic versatility. Among the tested microorganisms, *Nocardiopsis valiformis* and *Nocardiopsis alba* were particularly promising, as both exhibited positive activity for all four enzymes. These findings indicate that actinomycetes associated with the alkaline–saline Lonar ecosystem can serve as valuable candidates for the exploration of extracellular hydrolytic enzymes.

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