Isolation, Partial Characterization And Extraction Of Alkaline Protease From Bacterial Isolates Of Lonar Lake

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Introduction:

Lonar crater, is unique ecosystem situated in the Buldhana District of the Maharashtra State, India (Latitude 19˚58', Longitude 76˚36'). Lonar lake is a national geo-heritage, saline soda lake in basaltic rock with pH 9.5 to 10.5 (Frederickson et al., 1973). Extracellular enzymes like amylase, lipase, protease and cellulose are produced by *Bacillus cereus, Bacillus firmus, Enterococcus caseliflavus, Bacillus fusiformis, Bacillus cohnii, Bacilus horikoshi* isolated from water and sediment of alkaline Lonar Lake (Joshi et al., 2007).

Alkaline proteases are those which have the pH optima in the range of 8 to 11 and mainly belong to bacterial origin. Alkaline protease producers comprise many alkaliphilic, neutralophilic and alkalitolerent microbes. *Aeromonas hydrophilia, Bacillus licheniformis, Bacillus megaterium, Bacillus clausii and Bacillus subtilis are industrially important bacterial alkaline protease producers* (Sandhya et al., 2005).Starch degrading amylase enzymes are most important in the biotechnology industries with huge application in food, fermentation, textile and paper. Many microorganisms are able to produce amylases including *Bacillus sp*., *Lactobacillus, Escherichia, Proteus, Streptomyces sp., Pseudomonas sp.*etc.

Materials and methods:

Isolation and Identification of bacterial isolates:

Isolated bacteria collected from Lonar lake, Maharashtra, Grown & maintained on Horikoshi B medium having pH 12. The pH is maintained by 1 N NaOH. On basis of morphological, biochemical analysis were performed.

Production, extraction and confirmation of enzymes:

For proteolytic and amylolytic activity, isolates inoculated on alkaline skim milk agar and alkaline starch agar was used having pH 12(maintained by 1N NaOH), incubated at 37^{o}C for 48-72 hours. For extraction of crude enzyme protease two isolates inoculate in broth containing 1% casein & incubate at 37°C for 48 hours in shaking condition. Centrifuged at 3000-5000 rpm for 15 min. Clear supernatant serve as crude enzyme, and enzyme activity was checked by zone of solubalization around the well on skim milk agar plate.

Result and Discussion:

In the present study, total 3 samples comprising of 2 water and 1 sediment samples were collected from alkaline lonar lake. From these samples, 8 morphologically different colonies were isolated. Identification of isolates were based on cultural, morphological, and biochemical characteristics.

1. **Screening of isolates for proteolytic & amylolytic activity:** Out of 8 bacterial isolates 3 were a found to be positive for casein hydrolysis& starch hydrolysis. The zone of casein hydrolysis given by isolates were 23mm and 25mm respectively. The zone of starch hydrolysis was found to be 5mm each by isolates.

2. **Production & confirmation of crude enzyme :** After centrifugation, the clear supernatant pour into well containing skim milk agar plates. After 48 hours incubation zone of solubalization indicates that crude enzyme may be protease.
3. **Conclusion:**
   From above results, it was concluded that the bacteria were present in Lonar lake were grown only in alkaline conditions. These isolates were efficient for production of alkaline protease and amylase. The optimum pH required was 12, the optimum time required is 48-72 hours, and optimum temperature was 37^{o}C. The crude enzyme extracted from the isolate code shows maximum zone of solubalization around well on skim milk agar plates which indicates the crude enzyme may be protease.

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