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PRODUCTION, EXTRACTION AND CHARACTERIZATION OF COLD ACTIVE AND SALT STABLE ALKALINE PROTEASE FROM *HALOMONAS* SP. LAP520: LONAR SODA LAKE ISOLATE

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Abstract

Efficient cold active and salt stable alkaline protease producer was isolated from Lonar lake of Maharashtra, India. It was identified as *Halomonas* sp. LAP520 based on morphological and microscopic characters and enzyme profile. Casein yeast extract medium was used for alkaline protease production. 1050 U/mg production of alkaline protease was recorded from *Halomonas* sp. LAP520. Optimum catalytic activity of alkaline protease from *Halomonas* sp. LAP520 was recorded at 10 ^{o}C, pH 10 and 8 % NaCl. Therefore LAP520 alkaline protease can be used in different biotechnological industries.

Keywords: salt stable, cold active, alkaline protease, Lonar lake, soda lake.

Introduction

Alkaline proteases (E.C.: 3.4.11-19) are the most versatile industrial enzyme that execute a wide variety of functions and have tremendous important biotechnological applications in laundry detergents additives, leather finishing, silk industry, feeds modification, food processing, brewing, pharmaceuticals, diagnostic reagents, peptide synthesis, silver recovery from X-ray/photographic film, preparation of organic fertilizer and waste treatment (Anwar and Saleemuddin, 1997). To compensate energy related problems, cold active alkaline proteases were incorporated into detergents by some Asian and other countries where washing temperature was lower than 20-30°C. The primary habitats for occurrence of alkaline protease producers are alkaline habitats viz. soil and water of soda lakes, soda deserts and alkaline springs (Horikoshi and Akiba, 1982). Therefore we have selected the hyper alkaline and saline Lonar crater (19°59 N, 76°31 E) from Maharashtra state, India. This lake harbors many industrially important polyextremophiles from different phyla and genera. *Halomonas* sp. was reported by many researchers as predominant species in Lonar lake water and soil samples (Kanekar et al., 2008). However, very few species from genus *Halomonas* are known to produce alkaline protease. Some other alkaline protease producers reported from Lonar lake are *Bacillus subtilis, Bacillus pseudofirmus, Bacillus licheniformis* and *Bacillus cohnii* (Antony et al. 2013). In this regard, we have isolated *Halomonas* sp. from Lonar lake. Production and characterization of partially purified alkaline protease from *Halomonas* sp. was attempted.

Materials and methods

Isolation, Screening and Identification

To perform selective isolation of the species from the *Halomonas* genus, a composite water sample from the Lonar lake was spread on Halomonas agar, HSC agar, MH agar and AOL agar plates (pH 10.0, 5 % NaCl) and these plates were incubated at 10°C for 10 days in an incubator. Morphologically distinct colonies from afore-mentioned media were selected and cultivated on agar slants of the same media. Morphologically distinct isolates were spot inoculated onto alkaline skimmed milk agar (pH 10.0; NaCl 5%) plates and incubated at 10°C for 10 days to screen for extracellular alkaline protease production (Atlas 2005). Morphological, microscopic and physiological characters, enzyme profile, sugar utilization and antibiotic susceptibility pattern of the selected isolate were compared with the standard reference strains as described in 'Bergey's manual of systematic bacteriology' and selected isolate was identified (Sneath et al., 1986; Sharma et al., 2009; Khan et al., 2009; Vos et al., 2009; Palsaniya et al., 2012; Pathak and Sardar, 2012; Pathak et al. 2012, 2014; Pathak et al., 2015a,b,c,d,e,f; Khairnar et al., 2012; Kolekar et al., 2013; Hingole and Pathak, 2013; Pathak and Rathod 2013, Sardar and Pathak, 2014; Pote et al., 2014; Polkade et al., 2015; Dastager et al. 2015; Sharma et al., 2015; Jadhav and Pathak, 2015; Pathak and Rathod, 2015, 2016a, b).

Production, extraction and partial purification of alkaline protease

Fresh culture of the selected isolate (5 % v/v) from Lonar lake having inoculum size 10^5 cfu/mL was inoculated in 500 mL of casein-yeast extract production medium (pH 10) and incubated at 10°C and 120 rpm agitation speed for 5 days. The cell-free supernatant was collected after centrifugation of the fermented media at 10,000 rpm for 10 min and fractionated with a 60% saturated ammonium sulfate solution. The enzyme precipitate was collected and dissolved in Britton and Robinson buffer (pH 10.0) and dialyzed against the same buffer, and was considered as partially purified alkaline protease. The dialysate was assayed for quantitative alkaline protease activity according to the modified Anson's method. One proteolytic unit (U) was defined as the amount of the enzyme that releases 1 µmole of tyrosine per minute, under assay conditions. Total protein content each of the dialyzed samples was determined using bovine serum albumin as standard (Rathod and Pathak, 2014a,b; Lowry et al., 1951).

Characterization of the partially purified alkaline protease

Catalytic activity of partially purified alkaline protease was determined at pH range 6 to 12 by using Britton and Robinson buffer and at temperature range 10 to 40 ^{o}C. The optimum pH and temperature for catalytic activity of partially purified alkaline protease was determined. Catalytic activity of alkaline protease from the selected isolate was recorded at 2 to 10 % NaCl (Bhunia et al., 2012; Bhunia et al. 2013a,b; Pathak and Rathod, 2014; Pathak and Sardar, 2014; Rathod and Pathak, 2014a,b).

Results and discussion

Isolation, Screening and Identification

Among the different media used, maximum number of colonies was recorded from Halomonas agar. In average 109 colonies were appeared on Halomonas agar plates and 5 morphologically distinct colonies were selected to screen for alkaline protease production. These isolates were designated as LAP520 to LAP524. After screening, LAP520 isolate showed remarkable zone of clearance and

therefore selected for further studies. Colonies of LAP520 were smooth, round, off white and semi raised. Cells were observed motile. Cells were observed as short to slightly curved elongated rods. LAP520 was Gram stain negative and non spore former. Among the different 21 sugars tested, LAP520 was positive for dextrose, lactose, sucrose and fructose. LAP520 has shown optimum growth at 10 ^{o}C and pH 10. LAP520 has shown optimum growth at 5 % NaCl in medium. LAP520 produced extracellular protease, urease and lipase. LAP520 has shown sensitivity to Tetracycline, streptomycin and Ampicillin (25 and 30 µg/disc). LAP520 was identified as *Halomonas* sp.

Production of alkaline protease

1050 U/mg production of alkaline protease was recorded from *Halomonas* sp. LAP520.

Characterization of the partially purified alkaline protease

Catalytic activities of partially purified alkaline protease from *Halomonas* sp. LAP520 were recorded at pH range 6, 7, 8, 9, 10, 11 and 12 as 102, 204, 405, 450, 445, 540 and 520 U/mL respectively. Catalytic activities of partially purified alkaline protease from *Halomonas* sp. LAP520 were recorded at 10, 20, 30 and 40 ^{o}C as 540, 535, 510 and 200 U/mL respectively. The optimum temperature and pH for catalytic activity of partially purified alkaline protease from *Halomonas* sp. LAP520 was recorded as 10 ^{o}C and pH 10 respectively. Catalytic activities of partially purified alkaline protease from *Halomonas* sp. LAP520 were recorded at 2, 4, 6, 8 and 10 % NaCl as 342, 443, 502, 556 and 520 U/mL respectively. Maximum catalytic activity was recorded at 8 % NaCl.

Conclusions

Present study mark the significance of the naturally occurring and mesmerizing extreme alkaline habitat of Lonar soda lake, India for the presence of cold active and salt stable alkaline protease producers. The isolate *Halomonas* sp. LAP520 was belonged to gamma proteobacteria. Maximum catalytic activity of alkaline protease from *Halomonas* sp. LAP520 was recorded at 10 ^{o}C, pH 10 and 8 % NaCl. Therefore LAP520 alkaline protease can be used in different biotechnological industries.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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