International Journal for Multidisciplinary Research
International Conference on Multidisciplinary Research & Studies 2023
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Screening, Identification and Optimization of Protease Producer Bacillus Subtilis from Sewage

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Abstract

Different type of bacterial species has ability to produce protease enzyme which has various application in biotechnological areas. In the present investigation, *Bacillus subtilis* was isolated from sewage sample and identified on the basis of morphological and biochemical tests. Further, it was screened for protease production. The results showed that among the different carbon sources used, fructose was found to be the optimum carbon source whereas tryptone was found to be the optimum nitrogen source for protease production. Similarly, optimum pH and temperature was found to be 7.5 and 50°C respectively, for protease production.

Keywords: *Bacillus subtilis*, optimization of protease, protease production, sewage

Introduction

Proteases are the class of hydrolases enzymes that breakdown the peptide bonds of amino acids of a protein and has application in different type of industries such as leather, detergent, pharmaceutical, photography, food and agricultural industries. Proteases represent one of the three largest groups of industrial enzymes (Verma et al. 2011; Sathyavrathan & Kavitha 2013).

Proteases accounts for about 60 % of the total enzyme sales in different market sectors. The application of bacterial proteases is more significant when compared to the proteases from other sources like fungal and human. To supply adequate industrial proteases to meet the demand, investigation of the cost-effective way of enzyme production is very important which has industrially importance. Microbial proteases are preferred over the enzymes from plant or animal sources because of their wide-range biochemical diversity, their rapid proliferation, the limited space required for cell cultivation and the convenience with which the enzymes can be genetically manipulated to generate new enzymes for various application. Different types of microorganisms have potential to produce protease enzyme such as mold, yeast, bacteria etc. for the industrial application. However, among various bacteria, Bacillus sp. was found to be the major group that produces protease enzymes (Maugh, 1984; Gupta et al., 2002).

Sewage is generated by residential, institutional, commercial and industrial establishments and includes household liquid wastes from baths, toilets, kitchens and sinks that are disposed off via sewers. The one of the main organic contaminants in sewage is proteins. The bacteria present in sewage have potential to produce the protease enzymes which hydrolysis the protein. Such bacteria may be used for protease production (Sonune and Garode, 2015; Sonune et al., 2018). Hence, the present study focuses on screening, identification and optimization on protease producing Bacillus subtilis from the sewage effluent.

Materials and method

Sample collection and isolation of bacteria: The sewage sample was collected from drainage from Dhamangaon Rly. by standard procedure given in APHA and was subjected for serial dilution. The last dilution of sample was spread on plates containing nutrient agar by spread plate method. Plates were incubation at 37^0C for overnight for 24h at incubator. The different colonies from plates were purified and sub-cultured on nutrient agar slant and maintained at 4^0C in refrigerator for further study.

Screening and identification of proteases producing bacteria: All purified bacterial isolates were streaked on the skim milk agar plate and incubated at 37°C for 48 hours. Based on the zone of clearance, the high yield protease strain was selected for further studies. The bacterial isolate which showed maximum

zone of hydrolysis was examined for morphology characters such as Gram staining, spore staining, motility and biochemical tests such as Indole production, methyl red, Vogues Proskauer's, citrate utilization, triple sugar iron, nitrate reduction, urease, catalase, oxidase, sugar fermentation such as glucose, lactose, mannitol, sucrose and trehalose.

Enzyme production and assay:

Protease producing bacterial isolate was inoculated in skim milk broth (2% skim milk, 1% tryptone, 0.5% sodium chloride, 0.5% yeast extract) aseptically and was incubated at 37^0C for 48 hrs. shaker. After incubation, by using Whatmann No.1 filter paper cultured medium was filtered aseptically. The filtrate was subjected to centrifugation at10,000 rpm for 10 minutes. The supernatant was used as crude enzyme for estimation of proteolytic activity. 1% casein in 0.1 M phosphate buffer and pH 7.0 was used as substrate. Enzyme (1ml) and substrate (1ml) was incubated at 50^0C for 60 min. The reaction was stopped by adding 3ml Trichloroacetic acid. One unit of protease activity was defined as the increase of 0.1-unit optical density at 1hr incubation period. After this, it was centrifuged at 5000 rpm for 15 min. From this, 0.5ml of supernatant was taken and in this 2.5ml of 0.5 M sodium carbonate was added, mixed well and incubated 20 min. Now, 0.5ml of folin phenol reagent is added and the absorbance was read at 660 nm using spectrophotometer. The amount of protease produced was estimated and expressed in microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity (Lowry et al. 1951).

Optimization for protease production: The effect of different parameters such as pH, temperature, carbon and nitrogen sources were tested for protease production by the isolate. The effect of pH for amylase production was determined by culturing the bacterial isolate in the production media with different pH such as 6.0, 6.5, 7.0, 7.5 and 8.0. The enzyme assay was carried out after 48 hours of incubation. The effect of temperature on amylase production was studied by the incubating the culture media at various temperatures such as 25, 37, 45 and 55^0C. The enzyme assay was carried out after 48 hours of incubation. Similarly, the effect of different carbon sources such as maltose, fructose and sucrose were studied by replacing the original carbon source of the medium with equivalent carbon amount of each of the tested carbon sources. Similarly, different nitrogen sources such as skim milk, beef extract and tryptone were studied by replacing the original nitrogen source of the medium with equivalent nitrogen amount of each of the tested nitrogen sources.

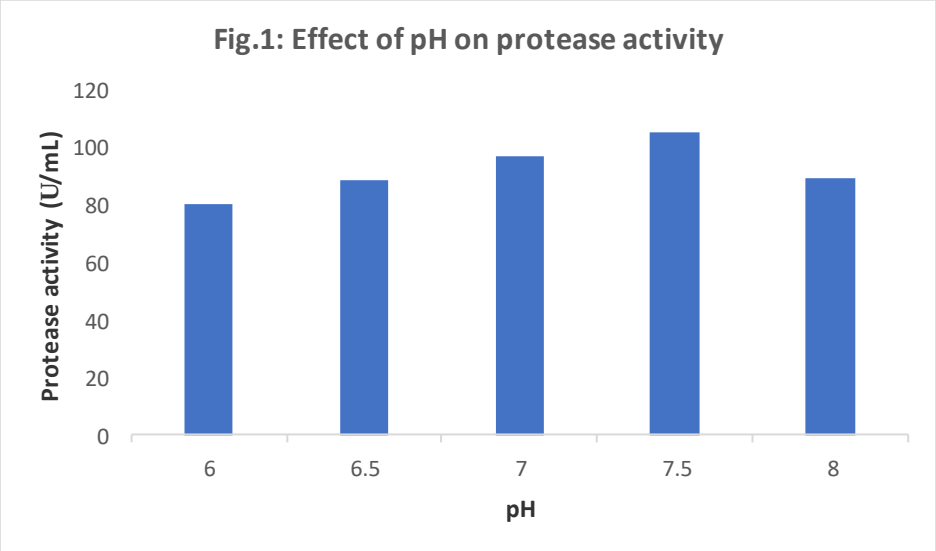
Results and discussion

Isolation, screening and identification of protease producer bacteria

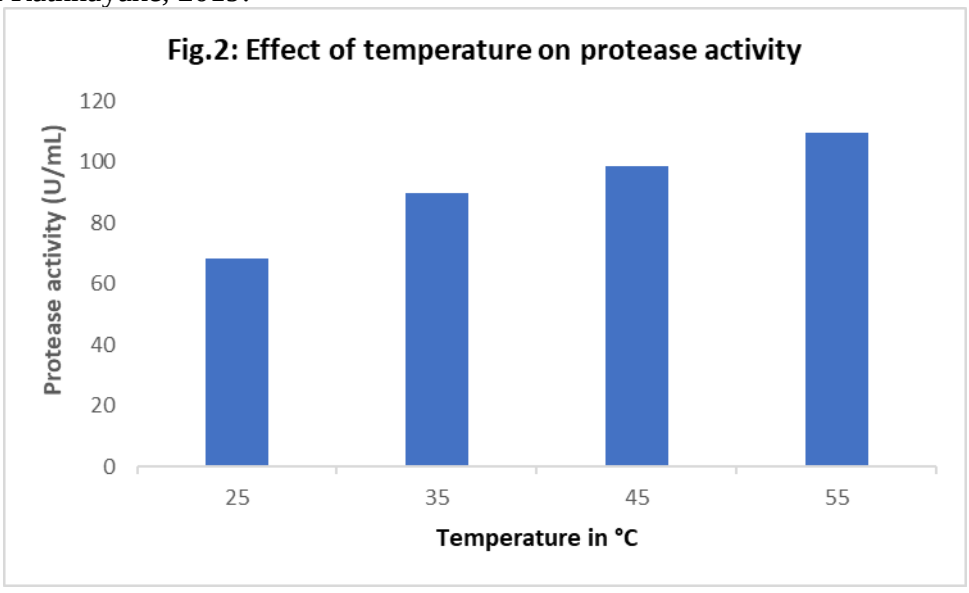
From the isolated bacterial strains on nutrient agar from sewage samples, total 15 bacterial strains showed protease producing ability on skim milk agar. Among them, one bacterial strain was found to be maximum protease producer. Further it was identified as *Bacillus subtilis* based on morphological and biochemical tests. Many studies reported the on isolation of enzyme producing bacteria from sewage (Garode and Sonune, 2013; Garode and Sonune, 2014).

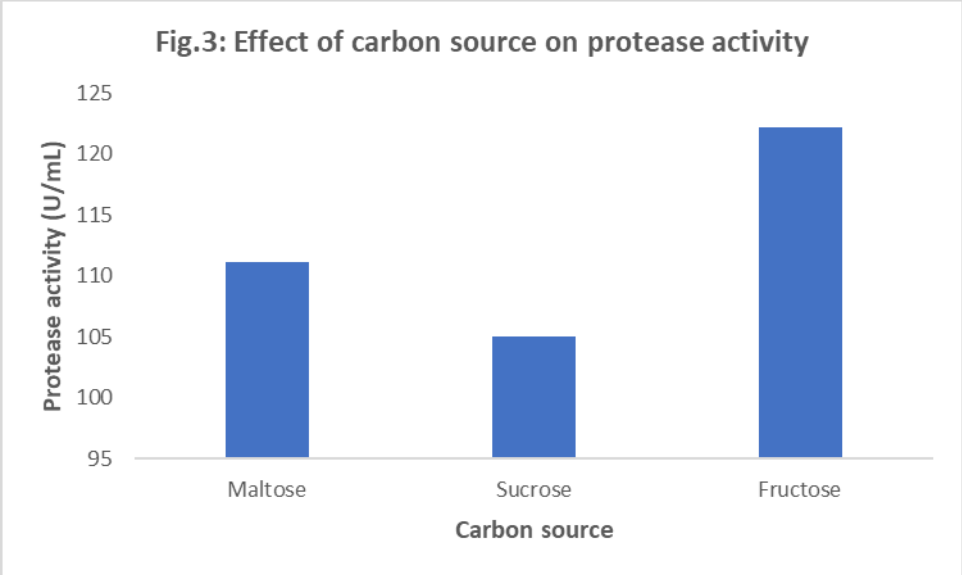
Optimization of protease production

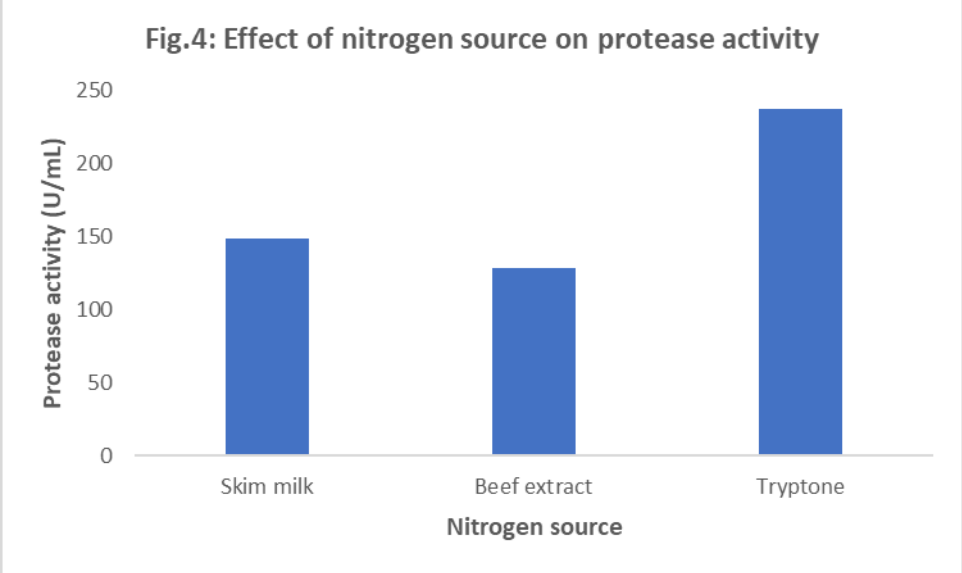
Culture conditions are strongly influenced the production of extracellular protease by bacteria. Hence it is very necessary to optimize cultural conditions. The pH of the medium strongly affects many biochemical processes and ions transport across the cell membrane (Moon and Parulekar 1991). The present results showed that maximum activity was found at pH 7.5 (105.41U/mL), above and below pH 7.5 the activity was found to be reduced. Many reports showed that maximum activity of protease was from neutral to alkaline Conditions (Thys & Brandelli, 2006; Refai *et al*., 2005; Nam *et al*., 2002).



The activity of the enzyme was measured between 25 to 55^0C temperature and the results suggest that as temperature increases, the enzyme activity was found to be increased. The maximum activity was found at 55^0C (109.64 U/mL). It suggests that probably the enzyme was moderately thermophilic. Our finding is comparable with Sevine et al., 2011 and Muhammad et.al., 2013. Different carbon sources like maltose, fructose and sucrose were tested for maximum activity of enzyme. The present results showed that maximum activity was found to be 122.14 U/mL when carbon source was fructose. Present result is similar with the results of Dissanayaka and Rathnayake, 2019. Different nitrogen sources like skim milk, beef extract and tryptone were tested for maximum activity of enzyme. However, the maximum activity was found when nitrogen source was tryptone. Present result is in contrast with Krishnaveni et al., 2012 and Dissanayaka and Rathnayake, 2019.







Conclusion:

The present investigation was based on optimization of production of protease by *Bacillus subtilis* isolated from sewage sample. We conclude that Protease from *Bacillus subtilis* showed optimum production at pH 7.5 and 55˚C temperature. Similarly, the maximum production was achieved when fructose as carbon source and tryptone as nitrogen source used in the media. The protease may become attractive alternative of available proteases in local detergent and leather industry and can play a vital role in the economic growth of the country.

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