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ALKALINE PROTEASE PRODUCTION BY *PSEUDOMONAS AERUGINOSA* ISOLATED FROM THE GUT OF *PILA OVATA*

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Abstract

Pseudomonas aeruginosa isolated from the gut of a freshwater snail (*Pila ovata*) was tested for its ability to produce the protease enzyme. The effect of different production parameters such as temperature, pH, carbon and nitrogen sources and sodium chloride concentration for protease production by the isolated bacterial strain was studied. The optimum conditions observed for protease production were temperature of 37^{o}C, pH 9, 1% glucose, 0.5% beef extract and 3.0% sodium chloride. Under optimized parameters maximum protease activity was 432U/ml. This bacterial isolate has potential that could be commercially exploited to assist in protein degradation in various industrial processes.
Keywords: Gut microbiota, freshwater snail, enzyme production, optimization.

INTRODUCTION

Proteases are enzymes that are widespread in nature. They breakdown proteins by hydrolysis of the peptide bond that exists between two amino acids of a polypeptide chain [1]. With the modern world focusing on eco-friendly products and product output, more and more chemical processes are being replaced by enzymatic methods.

Proteases can be classified according to their active pH range into neutral, acidic and alkaline proteases. Alkaline proteases are those enzymes that are active at alkaline pH with optimum pH in 9 to 11. Alkaline proteases are very important industrial enzymes, when compared to the other proteases, the alkaline proteases were found to be having more applications in various industries [2]. They are widely used in the film industry for recovery of silver from x-ray films, chemical industry for peptide synthesis, feed and food industry for production of protein hydrolysates, by waste processing companies, in the field of textile processing for degumming of silk and processing of wool and in the manufacture of detergents, pharmaceuticals and leather [1, 3, 4, 5].

Proteolytic enzyme producers are also helpful for the health of the ecosystems of this earth as these microbes decompose the dead and decaying animal or plant tissues in water or land. They can create pollution free environment and they are responsible for the recycling of nutrients [6].

Microbes serve as a preferred source of these enzymes because of their rapid growth, the limited space required for their cultivation and the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various applications [7]. The protease enzyme constitutes two thirds of total enzymes used in various industries and it accounts for about 60% of the total worldwide sale in the market [8].

In order to meet the industrial requirements as well as increasing demand of global enzyme market, isolation and optimization of enzyme production conditions of new promising strains should be a continuous process. Therefore, the present study is aimed at optimizing alkaline protease production by *Pseudomonas aeruginosa* isolated from the gut of a freshwater snail (*Pila ovata*).

MATERIALS AND METHODS

Sample collection, isolation and screening of potential protease bacteria

The specimens of *Pila ovata* were collected from Okpuhur Creek in Odhieke Community in Ahoada West Local Government Area of Rivers State, Nigeria. The snails were externally cleaned with ethanol and their gastrointestinal tracts dissected under sterile conditions. The gut contents were weighed and placed in a physiological solution and then diluted in a range 1:10 to 1:1000. Sub samples of 0.1ml of the dilutions were cultured on skim milk agar and incubated at 37^{o}C for 24-48 hours. Isolate which produced maximum zone of clearing was picked and streaked repeatedly on nutrient agar plates until pure. The purified isolate was identified based on its morphological and physiological characteristics [9].

Protease activity assay

The tested bacterium was inoculated onto a medium consisting of 1g peptone, 0.5g beef extract, 0.5g NaCl, 100ml distilled water and pH 7. It was incubated at 37°C for 24 hours. After incubation, the culture medium was centrifuged at 10,000 rpm for 15min to obtain the crude extract which was used as enzyme source for protease activity [10]. The crude extract was estimated for protease activity as per the method of (11) as described by (10) using casein as the substrate. The peptide, mainly tyrosine liberated during proteolytic digestion, was measured at 660nm in UV-Vis spectrophotometer.

Effect of temperature

The effect of temperature on protease production was studied by incubating the culture medium at three different temperatures 25°C, 37°C and 45°C for 24 hours. Protease activity was determined individually after 24 hours.

Effect of pH

The effect of pH on protease production was determined by culturing the bacterium in the protease production medium with different pH ranging from 6-11. The enzyme assay was determined individually after 24 hours of incubation at 37°C.

Effect of carbon and nitrogen sources

The effect of carbon and nitrogen sources on protease production was studied in the production medium with different carbon sources such as sucrose, glucose and maltose and nitrogen sources such as beef extract and yeast extract. They were added separately at the concentration of 1% for carbon sources and 0.5% for nitrogen sources. The enzyme assay was carried out individually after incubation at 37°C for 24 hours.

Effect of sodium chloride (NaCl)

The effect of NaCl on protease production was determined by growing the bacterium in the protease production medium with various concentrations of NaCl ranging from 1.5-3.5%. The enzyme assay was carried out individually after 24 hours incubation at 37°C.

Mass scale culture

Mass scale cultivation of *Pseudomonas aeruginosa* was carried out in protease production broth with optimized parameters such as temperature 37°C, pH 9, 1% glucose, 0.5% beef extract and 3.0% NaCl. The cells were harvested after incubation for 24 hours at 37°C by centrifugation at 10,000 rpm for 15min and the supernatant was used for enzyme assay.

RESULTS AND DISCUSSION

The study evaluated the protease producing ability of *Pseudomonas aeruginosa* isolated from the gut of freshwater snail, *Pila ovata*. It has been reported that bacteria of the digestive tract of aquatic animals participate with their enzymes in the process of degradation of nutrients [12].

The effect of temperature on protease production is shown in Fig. 1. The temperature of 37°C was found to be optimum with maximum of 365 U/ml protease activity. An increase in protease production with increase in temperature up to the temperature of 37°C for *Pseudomonas aeruginosa* from abattoir soil has been reported by [13] and [14] respectively. The decrease in protease production beyond 37°C reported by these researchers and also observed in this work proved that temperature plays a major role in enzyme production.

The effect of pH on protease production is shown in Fig. 2. Maximum protease activity (372 U/ml) was observed at pH 9. Similarly (10) and (14) reported a maximum alkaline protease production at pH 9. Similarly [10] and [14] reported a maximum alkaline protease production at pH 9 for *Pseudomonas aeruginosa* isolated from the gut of *Penaus monodon* and abattoir soil respectively. Bacterial alkaline proteases are characterized by their high activity at alkaline pH and broad substrate specificity [2].

The effects of carbon and nitrogen sources on protease production are shown in Fig. 3 and Fig. 4 respectively. Glucose supported the maximum production of protease enzyme while the best nitrogen source for protease production was beef extract. The results are in line with the findings of [15] and [10] who reported maximum enzyme activity in glucose medium among other carbon sources and beef extract among other nitrogen source for *Bacillus* spp. and *Pseudomonas aeruginosa* respectively.

The effect of NaCl concentration is shown in Fig. 5. Maximum protease activity (357 U/ml) was observed at 3.0% NaCl. This same result was also obtained by [10] who observed a maximum enzyme activity at 3% NaCl from *Pseudomonas* sp. isolated from the gut of *Penaeus monodon*. After

mass cultivation, the cell free extract of *Pseudomonas aeruginosa*, obtained by centrifugation, showed maximum protease activity of 432 U/ml. The growth and enzyme production of microorganisms are strongly influenced by medium components like carbon and nitrogen sources as well as cultural parameters like temperature and pH [2].

CONCLUSION

In this study, the appreciable high enzyme activity obtained during mass scale culture indicated that *Pseudomonas aeruginosa* isolated from the gut of freshwater snail (*Pila ovata*) is a potential producer of alkaline protease. Therefore, this bacterial isolate has potential that could be commercially exploited to assist in protein degradation in various industrial processes.

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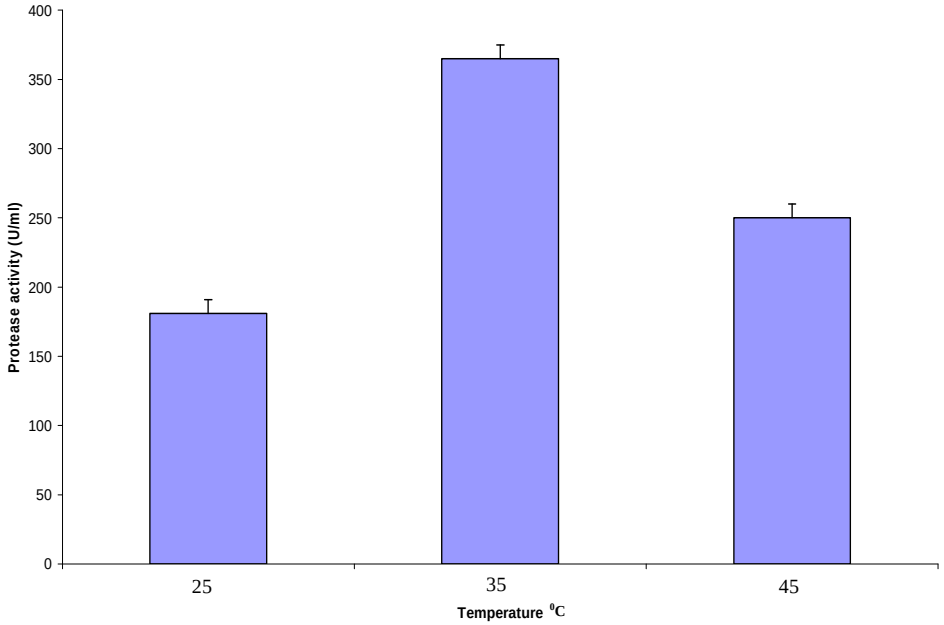

Fig. 1: Effect of temperature on protease production

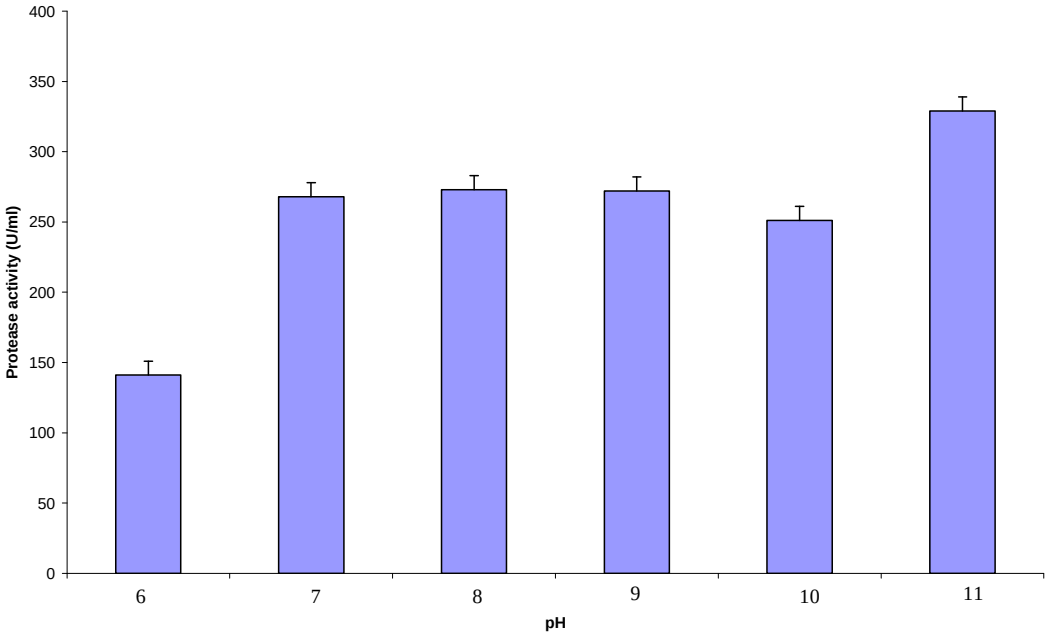

Fig. 2: Effect of pH on Protease production

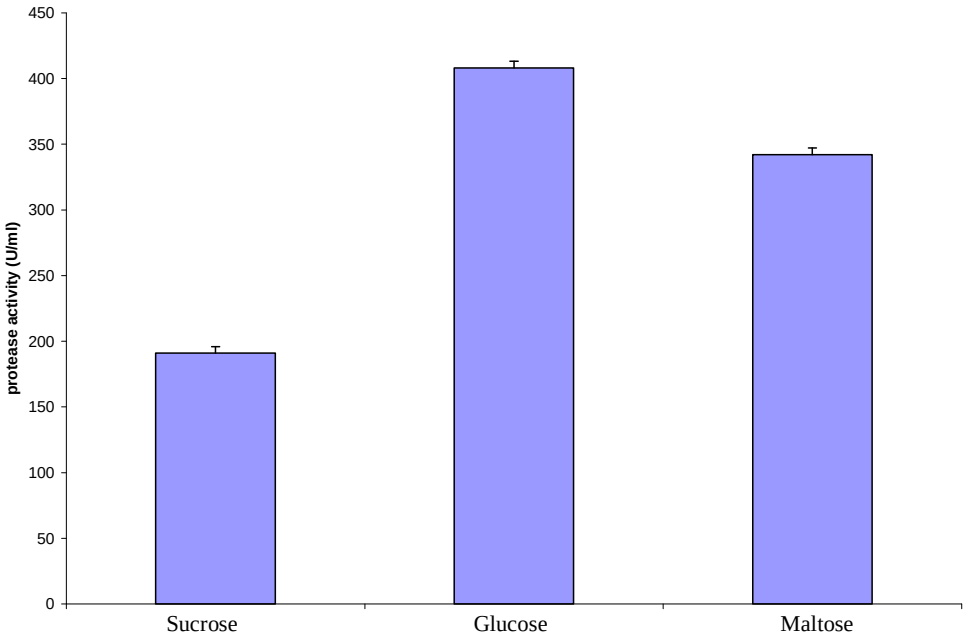

Fig. 3: Effect of carbon sources on protease production

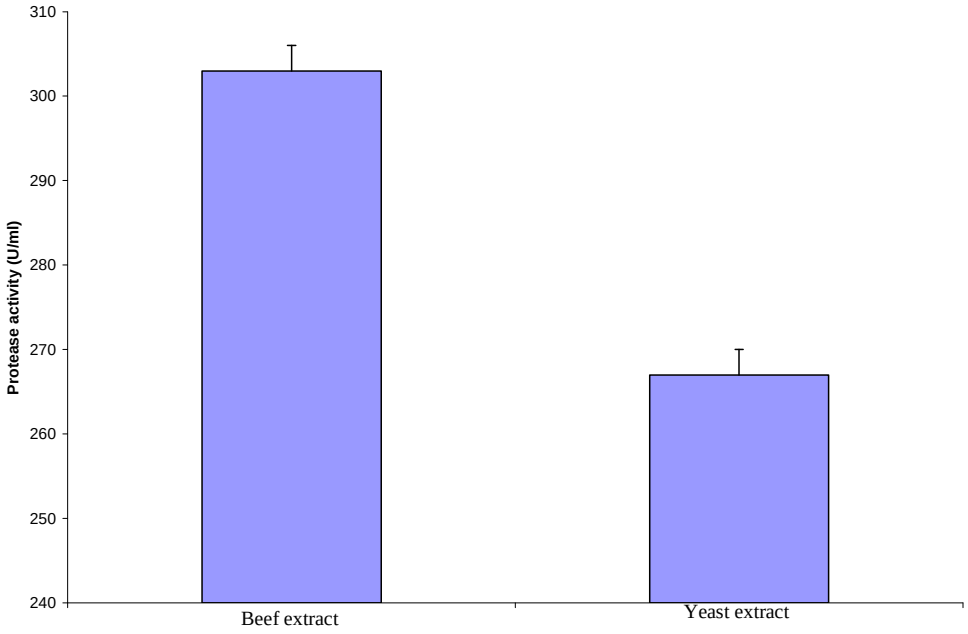

Fig. 4: Effect of nitrogen sources on protease production

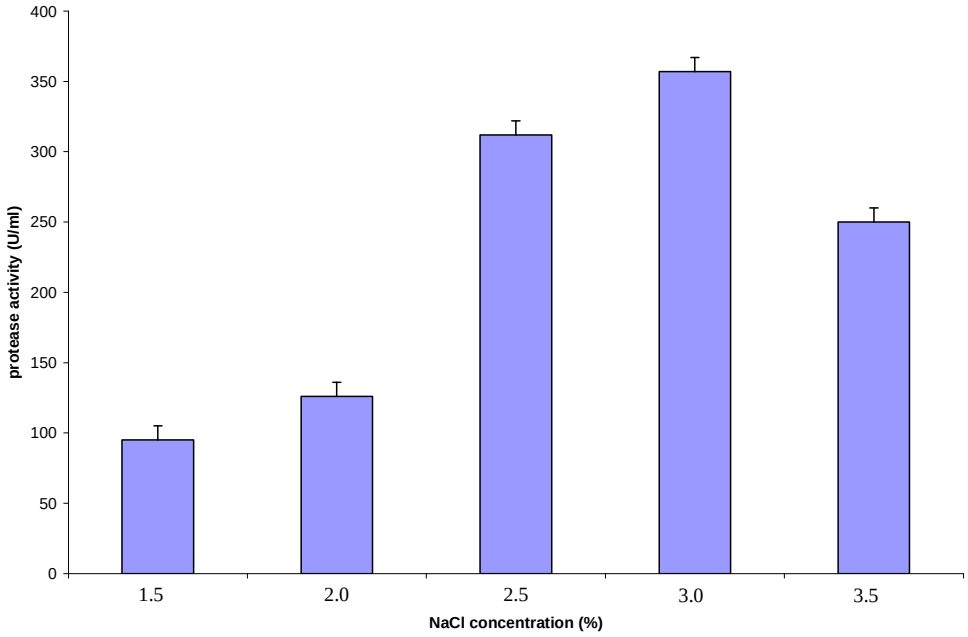

Fig. 5: Effect of NaCl concentration on protease production