IJSRM

INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY

An Official Publication of Human Journals

Human Journals

Research Article

July 2018 Vol.:10, Issue:1

© All rights are reserved by Mahmoud Suleiman Jada et al.

Isolation, Partial Purification and Characterization of Protease from *Bacillus Species* Obtained from Abattoir Soil Sample

IJSRM

INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY

An Official Publication of Human Journals

Mahmoud Suleiman Jada*[1], Abdullahi, Umar[2], Wurockekke, Abdullahi Usman[3], Dickson, Papa'a[4]

[1]*Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State.*

[2]*Department of Science Laboratory Technology, Modibbo Adama University of Technology, Yola, Adamawa State.*

[3]*Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State.*

[4]*Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State.*

Submission: 20 June 2018

Accepted: 27 June 2018

Published: 30 July 2018

www.ijsrm.humanjournals.com

Keywords: Purification, Characterization, *Protease* and *Bacillus species*

ABSTRACT

Isolation, partial purification and characterization of *protease* extracted from *Bacillus Species* isolated from abattoir soil sample is the aim of this study. Production medium for *Bacillus Specie*s was optimized by using different pH and temperature. *Protease* was partially purified by ammonium sulfate precipitation and ion exchange chromatography using DEAE cellulose. Ammonium sulfate yield 0.2 purification fold and specific activity of 0.003units/mg less than the crude extract and DEAE was able to separate the crude into different fractions with the most active fraction having a specific activity of 0.008 units/mg and purification fold of 0.533. The *protease* enzyme was highly active and stable from pH 4.4 to 5.4 with optimal activity at pH 5.0 and optimal temperature at 30ºC. This study clearly revealed that *Bacillus Specie*s isolated from abattoir soil sample is a potential producer of protease and DEAE cellulose is an effective and simple means of protease purification.

1.0 INTRODUCTION

Isolation is a chemical process of extracting a single or few enzymes from a complex mixture. This permits the substrate identification and comprises linking the enzyme and substrate such that the product is produced, which remains linked to the enzyme and extracting the enzyme by selectively isolating the product linked to the enzyme [1].

Characterization of an enzyme is the grouping of the enzyme according to their morphological, structural, and chemical features. Characterization is based on the high degree of specificity for their substrates and rate acceleration. Most enzymes function in the cellular environment at mild conditions of temperature, pH, and salt. Few non-biological catalysts can be so efficient in this type of environment. Enzymes play a critical role in everyday life [2].

Proteases are the complex multi-enzyme system, which catalyzed the hydrolysis of the amide bond in protein molecules; hence, it has been used in the field of textile processing for degumming of silk and processing of wool [3]. With the advent of new frontiers in biotechnology, the spectrum of protease application has expanded into many new fields, such as clinical, medical and analytical chemistry [4].

Microorganisms are the most important sources for enzyme production. Selection of the right organism plays a key role in a high yield of desirable enzymes. Habitats that contain protein are the best sources to isolate proteolytic microorganisms [5]. The important protease producing bacterial are species of *Bacillus, Pseudomonas, Arthrobacter, and Serratia*.

Due to the commercial uses of *protease*, there is a high rate of interest in isolation of *protease* from various species of bacteria that produce a suitable property of enzyme use industrially and medically hence the need for this study.

2.0 MATERIALS AND METHODS

2.1 COLLECTION OF SAMPLE

Abattoir soil samples were aseptically collected from four (4) different locations from a depth of 6cm by scrapping the top layer at the abattoir in Jimeta, Yola North local Government, Adamawa State, Nigeria. The samples were brought to the Department of Biochemistry of the Modibbo Adama University of Technology, Yola in a sterile polyethylene bag. The samples were dried in a hot air oven at 37℃ for 1 hour and then allowed to cool at room

temperature. Soil sample (1g) each was added to conical flask containing 100ml each of distilled water. The flasks were considered as stock culture.

2.2 ISOLATION OF *BACILLUS SUBTILIS.* FROM SOIL SAMPLE

2.2.1 Serial Dilution

A series of the cultured tube containing 9ml of sterile water was arranged in a test tube rack. From the stock culture, the 1ml suspension was transferred aseptically to the first tube (10-^{1}) and mixed well, from the first tube (10^{-1}), 1ml of the suspension from the first tube was transferred to the second tube (10^{-2}), and also mixed well. Similarly, dilution of up to 10^{-1} was made (serial dilution technique), 0.1ml of suspension from each culture tube was sprayed on a sterile nutrient agar and was incubated at 37ºC for 24 hrs. Pure Culture was Stored at 4ºC in the refrigerator [6].

2.3 EXTRACTION OF PROTEASE FROM *BACILLUS SUBTILIS*.

The *protease* was produced in a fermentation media containing: NaCl 0.04g, peptone 0.20g, yeast extract 0.10g, starch 1g, NaOH 0.5. The media was sterilized by autoclaving at 121ºC for 15min. After cooling, the media was transferred in to test tube, inoculated with the bacterial media, and incubated at 37ºC for 24 hours. The raw extract of *protease* in fermentation media was separated from the bacterial cells by cold centrifuge at speed of 10000 rpm for 40min at 4ºC[7].

HUMAN

2.4 PROTEASE ACTIVITY ASSAY

Protease activity was assayed by a modified method of Xiong *et al.*, [8] using casein as a substrate. Casein solution (1g) was prepared in 0.1M citrate-phosphate buffer (pH 5.4) and used as the substrate; the solution was heat-denatured at 100ºC for 15 minutes and then allowed to cool. The enzyme extract (1ml) was thoroughly mixed with the substrate solution (10 ml) and the reaction mixture was incubated at 28^{0}C for 1 hour. The reaction was terminated by the addition of 3ml of cold 10% trichloroacetic acid (TCA) and the tubes were allowed to stand for about 1 hour at 2ºC to allow precipitation of undigested protein, The color develops after adding 1.5ml of 3 – fold diluted folin – ciocalteu reagent in a dark environment for 30min. For the control of experiments, 0.5ml of the enzyme extract was first incubated at 28ºC for 1 hour before the addition of TCA and 1g casein. The reaction mixture

was centrifuged at 10000rpm for 5min at 4°C. Optical density readings of the decanted supernatant were measured at 660nm. One unit of protease activity was defined as the amount of enzyme that released 1ug tyrosine per ml per minute for casein under the specified assay conditions [9].

2.5 PURIFICATION OF ENZYME

The enzyme was isolated by spinning the culture at low RPM for 10 mins to remove the cells. The extract was then purified using ammonium sulfate precipitation method, and ion exchange chromatography. The formulas below were used for the calculation of the total activity (units/ml), specific activity (units/mg), percentage yield (%) and purification fold.

A) Specific Activity (units/mg) $= \frac{\text{Total Enzyme Activity}}{\text{Total Protein}}$

B) Purification Fold $= \frac{\text{Specific Activity}}{\text{Specific Activity of the first}}$

C) Percentage yield (%) $= \frac{\text{Total Enzyme}}{\text{Total Activity}} \times 100$

HUMAN

2.6 AMMONIUM SULPHATE PRECIPITATION

The extract was precipitated for protease purification by the addition of solid ammonium sulfate up to 90% saturation level. Enzyme extract (0.5ml) was added to 1g of casein solution (10ml) and was allowed to stand for 1 hour and after which reaction was terminated by 10% TCA and allowed for 1 hour at 2°C. The follinidase reagent was added for color development for 30 min and read at 660nm [10].

2.7 ION EXCHANGE CHROMATOGRAPHY

The purification process of the enzyme sample was performed using Ion exchange chromatography system equipped with Fraction collector; Biologic LP Chromatography Systems (Biorad, USA). Enzyme extract (5ml) was applied to a diethylaminoethyl Cellulose (DEAE cellulose) column previously equilibrated with 1.0 M Tris-HCl buffer (pH 8.0) slowly percolating large volume of buffer through the packed material. The elution was accomplished with 15-column volume gradient 1.0 M Tris-HCl buffer pH 8.0. The flow rate

was controlled at 1.0 ml/min by 3 ml of fractions collected. After assaying the fractions for protease activity, fractions showing the highest activity was pool together and stored at -20 °C [8].

2.8 DETERMINATION OF PROTEIN CONTENT OF PROTEASE ENZYME

Protein content was determined using the Lowry method. After the enzyme activity was assayed, the result obtained was used to calculate protein content since one unit of protease activity was defined as the amount of enzyme that released 1ug tyrosine per ml per minute for casein under the specified assay conditions based on Lowry *et al.* [9].

2.9 DETERMINATION OF OPTIMAL pH

One gram of casein solution was prepared in 0.1M citrate-phosphate buffer at pH values of 4.0, 4.4, 5.0 and 5.4. The casein solution was then heat-denatured at 100ºC for 15min and allowed to cool. *Protease* assay was carried out using the prepared casein solution as substrate [11].

2.10 DETERMINATION OF TEMPERATURE OF THE PURIFIED ENZYME.

One-gram (1g) casein solution was prepared as described earlier. *Protease* assay was carried out by incubating 0.5 ml of the enzyme extract with 1ml of the substrate for 1 hour at 20, 30, 40 and 50ºC [12].

3.0 RESULTS

3.1 ISOLATION OF *BACILLUS* SPECIES

Among the four samples used, the plate labeled PAS4 exhibit better growth of *Bacillus specie*s on nutrient agar showing clear zone around the growth. The presence of white precipitate around the colonies indicates *Bacillus specie*s production (Plate 1). The organism exhibited proteolytic activity as shown in plate 2. Casein hydrolysis was carried out which confirm that *Bacillus Species* is a producer of protease enzyme as shown in plate 3.

Plate 1. **Isolated *Bacillus Specie*s from abattoir soil sample**

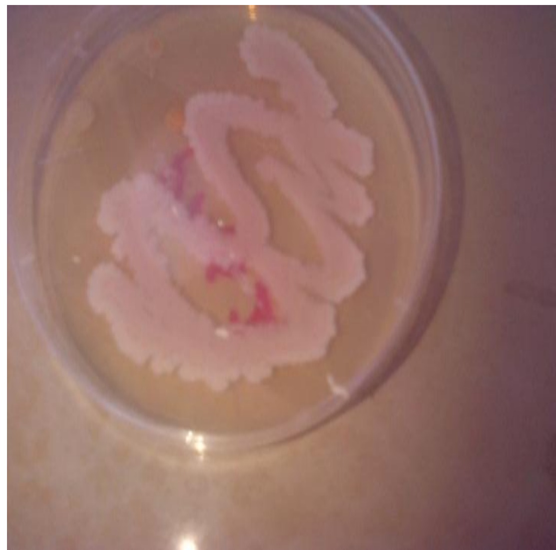

Plate 2. ***Bacillus Specie*s from abattoir soil sample showing proteolytic activity.**

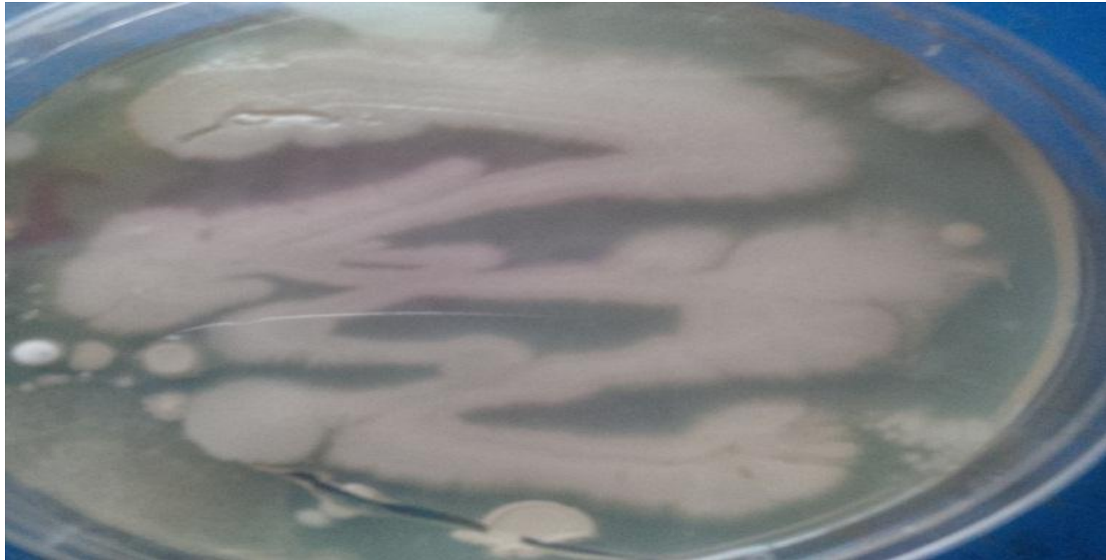

Plate 3. Confirmation of *Bacillus specie* as a Protease Producer

3.2 EXTRACTION OF PROTEASE FROM *BACILLUS SPECIE*

Protease extraction by submerging fermentation formed cloudy particles and some debris settling under. The extracted protease is shown in figure 1.

Figure 1. Extracted crude protease enzyme from *Bacillus species* isolated from abattoir soil sample.

3.3 PARTIAL PURIFICATION OF PROTEASE ENZYME.

Ammonium sulfate precipitation yields 0.2 purification fold and specific activity of 0.003(units/mg). Ion exchange using 3-DEAE cellulose separated into 4 fractions and fraction 3 has the highest activity of 0.008 (units/ mg) and 0.533 purification fold. Results are presented in table 1.

Table 1.Partial Purification of Protease from *Bacillus Species*

Fraction	Total protein (mg/ml)	Total activity (units/ml)	Specific activity (units/mg protein)	Yield (%)	Purification fold
Crude extract	16.890	0.25	0.015	100	-
Ammonium sulfate	14.050	0.045	0.003	18	0.200
DEAE cellulose					
Fraction 1	10.990	0.031	0.002	12.400	0.133
Fraction 2	7.590	0.038	0.005	15.200	0.333
Fraction 3	4.550	0.038	0.008	15.200	0.533
Fraction 4	2.030	0.026	0.012	10.400	0.800

HUMAN

3.4 Determination of Optimal Temperature.

Determination of temperature was carried out on protease activity using various temperatures for one hour at 20, 30, 40 and 50ºC. The maximum temperature of the enzyme is 30ºC. The results are represented in figure 2.

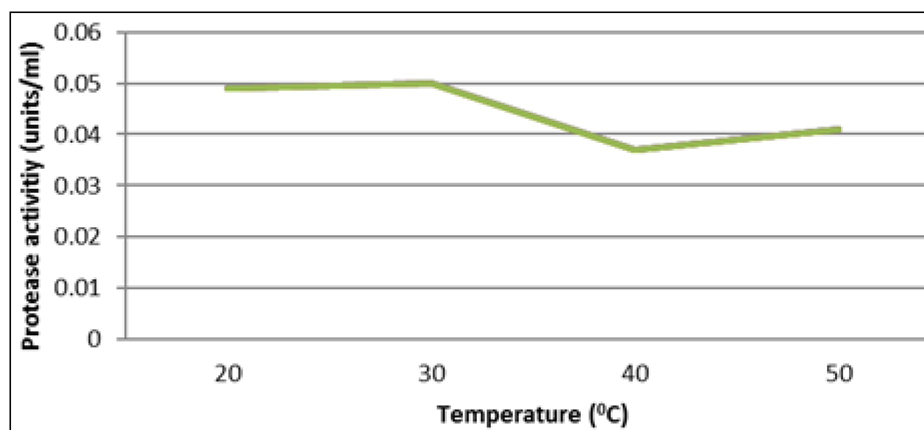

Figure 2: *Protease* activity against temperature

3.5 Determination of Optimal pH

Determination of pH was carried out on protease various pH 4.0, 4,4, 5.0, 5.4. The enzyme protease has an optimal pH at pH 5.0. The results are represented in figure 3.

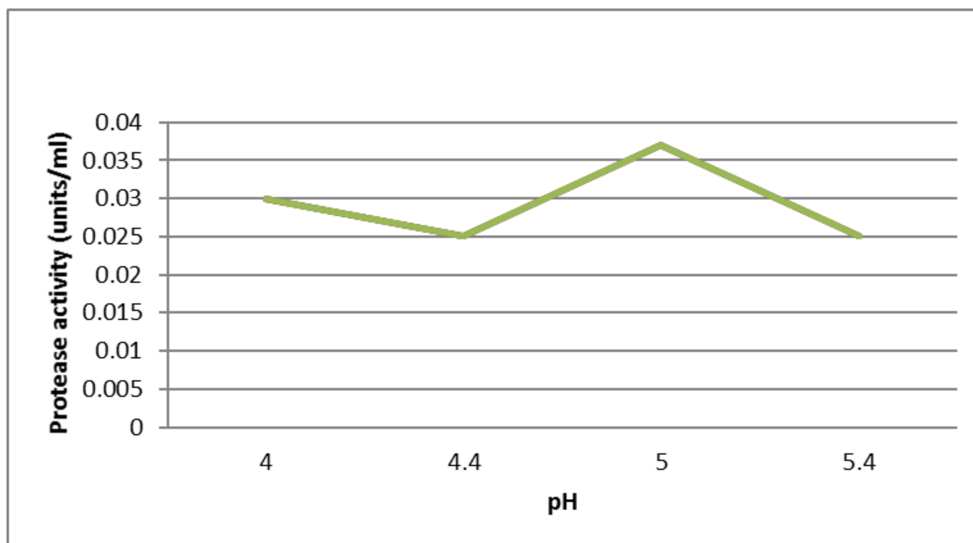

Figure 3: Protease activity against pH

4.0 DISCUSSION

This study revealed that *Bacillus* Species isolated from abattoir soil sample is a potential producer of *protease*. This suggested that soil samples could serve as a source of organisms for enzyme production. There are many microbial sources for producing protease but strains of *Bacillus Species* dominate the industrial sector [13].

Ammonium sulfate precipitation resulted in a 0.20 fold decrease in purification with the specific activity of 0.003 units/mg over crude enzyme (0.015 units/mg). This result shows that ammonium sulfate is not an effective means of protease purification extracted from *Bacillus specie*s isolated from Jimeta abattoir. This observation is in agreement with that of Charkaraborty *et al.,* [14] who also stated that ammonium sulfate is not an effective means of *protease* purification. Purification of protease using DEAE cellulose was able to separate the crude into the active fraction, which resulted in the observed increase in specific activity and purification fold where fraction 3 has the highest activity of 0.008unit/mg. With the result obtained, DEAE cellulose is an effective and simple means of protein purification. Rourke [15] also reported that DEAE cellulose is an effective means of protease purification.

The optimum temperature effect of *protease* from *Bacillus Species* isolated from Jimeta abattoir is 30ºC. As the temperature of the medium increases protease activity increases and decline after the optimal temperature which is 30ºC. In another study also reported that *Streptococcus lactis* and *Lactococcus lactis* both produce *protease* which has maximum activities at 28^{0}C and declined activities after 30ºC [16]. At temperature beyond 30ºC *Bacillus species* produced protease but in a lesser yield than that at optimal temperature. This is in accordance with the view of Daniel *et al.,* [17] who stated that increase in temperature led to increasing in activity but that there was the limit to the increase in activity because high temperature led to a sharp decrease in activity which could be due to denaturing of protein structure.

The pH of the reacting medium affects *protease* from *Bacillus species* isolated from Jimeta abattoir. Optimum activity was expressed at pH 5.0 this indicates an acidic *protease* nature. The catalytic activity of an enzyme is affected by the hydrogen ion concentration of its reaction medium and nature of amino acid found on it catalytic site [18].

In conclusion, the results obtained in this study have provided evidence for the *protease* producing ability of *Bacillus species* isolated from Jimeta abattoir. The influence of environmental factors on the *protease* production of the isolate was also evidence in this study. This study has given a hint that microbial wealth of *protease* producing bacteria isolated from abattoir environment can be harnessed for biotechnological processes, industries, textiles, and medicine.

REFERENCES

1. Woodley, J. M (2008). New opportunity for biocatalysis making pharmaceutical processes greener. *Trends Biotechnology Vol. 26 Pp. 321-327*
2. Valeika, V., Baleska, K and Sirvaityte, J (2012). Alkali-free method of hiding preparation for tannins. *Brazilian Journal of Chemical Engineering., Vol.29, n. 2, 315-323*
3. Ravel, C., Sauer, J., and Sigurdskjold BW, and Christensen U.(2005). Glucoamylase: Structure/Function, Relationships, and Protein Engineering. *Biochemistry and Biophysics Acta. Vol.1543, Pp275-293.*
4. Adinarayana, K., Jyothi, B. and Ellaiah, P. (2005). Production of Alkaline Protease with Immobilise Cell of *Bacillus Subtilis* P.E – 11 in various Matrices by Entrapment technique. AAPS PharmSciTech 2005; 6 (3) Article 48 (http://www.aapspharmscitech.org).
5. Dalev, P.G (1994). Utilization of Waste Feathers from Poultry Slaughter for Production of Protein Concentrate. *Journal of Bioresources and Technology. Vol. 48. Pp 265-267.*
6. Kumar, S., Wittenberg, N. J and Oh, S (2014). Purification and characterization of a thermostable alkaline protease from alkalophilic *Bacillus pumilus*. *Letters in applied microbiology* Vol. 34 Pp. 13-17.
7. Ishtiaq, A., Muhammad, A. Z and Hafiz, M., Nasir, I., (2011).Purification and Kinetic Parameters Characterization of an Alkaline Protease Produced from *Bacillus subtilis* through Submerged Fermentation Technique. *World Applied Sciences Journal; 12 Vol.6 Pp 751-757.*

8. Xiong, H., Song, L., XU, Y and Tsoi, M.Y (2007). Characterization of proteolytic bacteria from the aluentian soil microbiology and Biotechnology and their Proteases. *Journal of Industrial Microbiology*. Vol 34(1). Pp63-71

9. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.(1951). Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry*. Vol.193. Pp265-275

10. Dixon M. and Webb, E. (1971), Enzymes Second ed., Longmans and co. ltd, London, Great Britain.

11. Shanmugam, S., Satishkumar, T and Shanmugaprakash, M (2012). Enzyme Technology 2nd Edition. I.K. International Publishing House Pvt. Ltd. pp 154,185-186.

12. Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T, Singh, D and Mohan, R (2000). Advances in microbial amylases. *Biotechnology Applied Biochemistry*; Vol. 31, Pp.135-152.

13. Gupta R, Beg QK, Lorenz P. (2002): Bacterial alkaline proteases: molecular approaches and industrial applications. *Applied Microbiology Biotechnology***.** 59(1):15-32.

14. Charkraborty, R. and Srinivasan M.(1992). Production and regulation of thermostable Protease by *Pseudomonas sp*. B45. Acta Microbiologia Hungarica. Vol. 39.Pp 181- 191.

15. Rouke, K., Szweda, L., and Stadtman, E.,(2002). Effect of HNE Modification Induced by A.B Meprilyciene. *Internal Journal Vol. 2 Pp 30-31*

16. Akinkugbe AO, Onilude AA. (2013) Selective comparability and physiological studies of lactic acid bacteria protease and *Calotropis procera* (Linn) extracts. PeerJ PrePrints 1:e4v1 https://doi.org/10.7287/peerj.preprints.4v1

17. Daniel R.M., Peterson, M.E., and Danson MJ(2010). The molecular basis of the effect of Temperature on Enzyme Activity. *Biochemistry Journal., Vol.425(2). Pp 353-360.*

18. Voet, D., Voet, J.G and Pratt, C.W (2013). Principles of Biochemistry. John Wiley and Sons, Pp. 1077