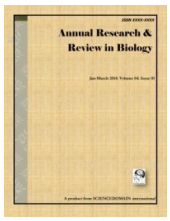

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Evaluation of *Spirogyra rhizobrachialis*, *Merismopedia elegans* and *Synedra spp* as Potential Sources of Proteases

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Authors' contributions

This work was carried out in collaboration between all authors. Author SEA designed the study, wrote the protocol and interpreted the data while author SAA managed the literature searches and wrote the first draft of the manuscript, while author SCU managed the laboratory analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To isolate and characterise the proteases from *Spirogyra rhizobrachialis, Merismopedia elegans* and *Synedra* species in order to evaluate them as potential sources of protease.
Study Design: Three *Algae* species were evaluated as potential sources of protease.
Place and Duration of Study: Department of Biochemistry, Ahmadu Bello University Zaria-Nigeria, between March 2013 and August 2013.
Methodology: A study on the potentials of *Algae* as source of proteases was conducted using three species of *Algae*, namely, *Spirogyra rhizobrachialis, Merismopedia elegans* and *Synedra* species which were identified and classified microscopically following fixation with Lugol solution. Proteases from these species were then characterised by determining their kinetic properties with respect to the rate of production of tyrosine at 660 nm using casein as substrate. The effects of Ca^{2+}, Mg^{2+}, Mn^{2+} and Hg^{2+} on the protease activity were monitored.
Results: Protease from *S. rhizobrachialis* had the most catalytic efficiency of 0.152 and most specificity to casein with a K_m value of 3.125g/L. The divalent cations; Ca^{2+}, Mg^{2+} were found to activate the enzyme from all three *Algae* species. Mn^{2+} served also as an activator for the protease in all but *Synedra spp.* where it inhibited the protease activity. In all three *Algae* species, Hg^{2+} was

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found to inhibit the enzyme.
Conclusion: The result shows that *S. rhizobrachialis is* a better source of protease amongst the three strains, and hence could add to the pool of microbial sources of proteases for industrial applications and other biotechnological processes.

Keywords: Protease; Algae; characterise; industrial; application; sources.

1. INTRODUCTION

Proteases are a group of enzymes that play an important nutritional and regulatory role in nature. They catalyse the hydrolysis of peptide and ester bonds. Based on the nature of the nucleophile in the hydrolysis reaction, they are broadly grouped into six catalytic types: serine, threonine, cysteine, aspartic, glutamic and metallo-proteases [1]. Proteases accounts for about 60% of the total industrial enzymes in the world market and approximately 40% of the total worldwide enzyme sale [2]. They are generally used in detergents [3], food industries, leather, meat processing, cheese making, silver recovery from photographic film, treatment of inflammations and virulent wounds [4]. The major enzymatic component in detergent is proteases which could induce unspecific autolysis and proteolysis of enzymatic components during storage [5]. The optimization of stabilization and inhibition of the supplemented proteases is nowadays an interesting research field [6-8].

Proteases are known to play some roles in pathogenesis, as alkaline proteases facilitate the invasion of the host cell by influenza A viruses through receptor mediated endocytosis [9]. Increases in protease expression and activity are associated with malignant progression and poor patient prognosis in a number of human cancers [10].

Proteases are found in a wide diversity of sources such as plants, animals and microorganisms but they are mainly produced by bacteria and fungi. Microbial proteases are preferred to plant and animal sources to various advantages like less production time. A variety of microorganisms such as bacteria, fungi, yeast and *Actinomycetes* are known to produce these enzymes [11]. Molds of the genera *Aspergillus, Penicillium* and *Rhizopus* are especially useful for producing proteases, as several species of these genera are generally regarded as safe [12]. Microbial proteases are degrading enzymes, which catalyse the total hydrolysis of proteins [13]. They are predominantly extracellular and can be secreted in the fermentation medium. They play an important role in biotechnological processes accounting for approximately 59% of the total enzyme used industrially [14]. Commercially, proteases can be produced and administered as food supplement to enhance digestion in systematic therapy [15].

Major hindrances to the exploitation of commercial enzymes are their yield, stability, specificity and the cost of production. New enzymes for use in commercial applications with desirable biochemical and physiochemical characteristics and low production cost have been focus of much research [16]. In this study, proteases were isolated from three different *Algae* species; *Spirogyra rhizobrachialis*, *Merismopedia elegans* and *Synedra spp* of the phyla Chlorophyta, Cyanobacteria and Baccillariophyta, respectively. The isolated proteases were characterized and the *Algae* with the most catalytic efficient protease was identified for possible industrial application.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The *Algae* samples used for this research were harvested from different locations in Zaria. Classification of the species was done under the microscope upon fixation with Lugol solution by considering the most prevalent species in the sample at the department of biological sciences Ahmadu Bello University Zaria, Nigeria.

2.1.1 Growing of the *Algae*

The *Algae* were grown in a bold basal media which is made up of three main components; stock solution, trace elements and soil extract. Stock solution was prepared in 40ml distilled water and is composed of sodium nitrate (1g), magnesium sulphate (0.3g), sodium chloride (0.1g), dipotassium phosphate (0.3g), mono potassium phosphate (0.7g) and calcium chloride (0.1g).

Trace elements were prepared in a 100ml solution composed of zincsulphate (0.88g), magnesium chloride (0.144g),manganese oxide (0.071g), copper sulphate(0.157g), cobalt nitrate (0.049g), boric acid (1.142%), ethylenediaminetetraacetate (5g), potassium hydroxide (3.1g), ironII sulphate (0.498g) and sulphuric acid (0.1%). The final volume was made up to 1 litre with distilled water.

Soil extract was prepared air drying humus enriched soil. 200 ml of soil was added to 400ml of tap water and autoclaved. This was allowed to settle over a few days and the supernatant soil extract was decanted.

The final medium was formed by combining 10ml of stock solution, 1ml of trace element solution and soil extract 250ml. Exactly 2ml of the various *algae* species were pipette into 2 ml of the final medium. This was kept under constant supply of light and at room temperature of 25±4℃ for their growth and shook every day for fourteen days [17].

2.2 Determination of Protease Activity

The assay was carried out by monitoring the rate of production of tyrosine at 37℃ for 30 minutes using casein as substrate in a method described by Carrie [18]. This is done with respect to the rate of production of tyrosine at 660 nm with casein serving as a substrate.

2.3 Effect of Substrate Concentration

The effect of substrate concentration on the protease activity at 0.35%, 0.45%, 0.55%, 0.65%, 0.75% and 0.85% of casein was monitored for *Spirogyra rhizobrachialis, Merismopedia elegans* and *Synedra* species as described by Carrie [18] to determine the kinetic parameters (K_m and V_m). The rate of production of tyrosine with respect to casein concentration at 37ºC and pH 7.5 was monitored.

2.4 Effect of pH on Enzyme Activity

The effect of pH on the protease activity at a pH range of 4-9 was monitored as described by Carrie [18] to determine the optimum pH for the protease activity. Here, rate of production of tyrosine with respect to variable pH at 37℃ was monitored, and pH with the highest activity corresponding to the optimum pH.

2.5 Effect of Temperature on Enzyme Activity

The activity profile of the purified enzyme was determined as a function of temperature to determine the optimum temperature suitable the enzyme's catalytic action at a temperature range of 20-60℃. Here, rate of production of tyrosine with respect to variable temperature at pH 7.5 was monitored, and temperature with the highest activity corresponding to the optimum temperature.

2.6 Effect of Activators and Inhibitors

To establish the effect of metal ion, (Ca^{2+}, Mg^{2+}, Mn^{2+} and Hg^{2+}) on crude enzymes, metal salt solutions were prepared in concentration of 10mM, and 1.0ml of metal solution was mixed with 5.0ml of crude enzymes and was incubated for 2hrs. Separate protease activities for free and incubated (with each divalent metal) crude extracts were determined.

2.7 Determination of Enzyme Parameters

The V_m and the K_m values for each protease were derived from the reciprocals of the Y and –X axes of the Lineweaver-Burke plot respectively. While the catalytic efficiency for each protease was derived from the ratio of V_m to K_m.

3. RESULTS AND DISCUSSION

3.1 Protease Activity

This study revealed all three *Algae* species to be potential sources for microbial proteases, having recorded certain level of activities. The data obtained for the protease activity of the three species of *Algae* revealed *S. rhizobrachialis* had the most activity of 0.330units/ml followed by *M. elegans* with 0.270units/ml and *Snedra spp.* having the least activity of 0.074units/ml.

3.2 Effect of Substrate Concentration

The Lineweaver-Burk plots for the protease from all three species are presented in Fig. 1, 2 and 3 for *S. rhizobrachialis*, *M. elegans* and *Synedra spp* respectively *S. Rhizobrachiali s*had a catalytic efficiency of 0.152 with V_m and K_m of 0.476units/ml and 3.125g/L respectively. *M. elegans* had catalytic efficiency of 0.115 with V_m and K_m of 0.370units/ml and 3.226g/L

respectively. *Synedra spp.* had the least catalytic efficiency of 0.039 with V_m and K_m of 0.141units/ml and 3.571g/L respectively. However, variation in the K_m values of the proteases for the three species gave an indication regarding their substrate specificities. Protease from *S. rhizobrachialis* has the most catalytic efficiency of 0.152 with the least K_m value of 3.226g/L, thus implying the most specificity of the proteins. The K_m values of 0.8g/L for alkaline protease from *A. Niger* [19] and 0.6g/L from *A. flavus* [20] were however reported.

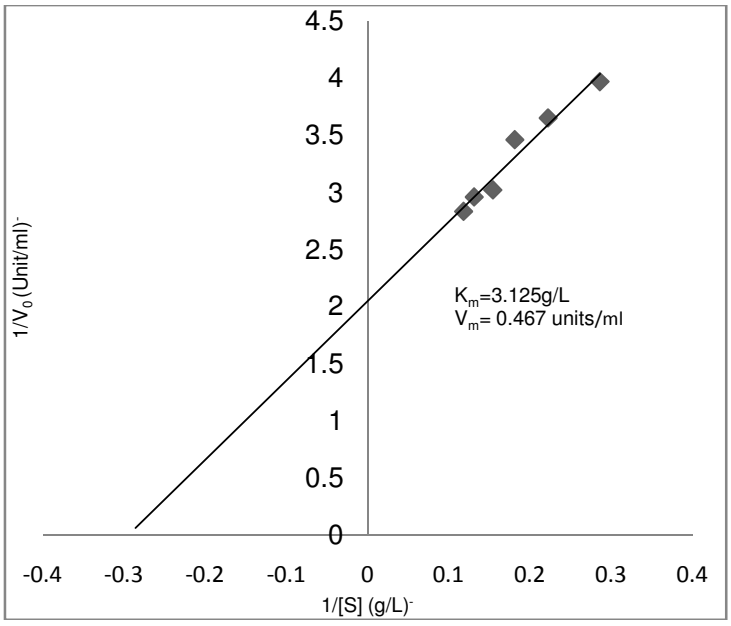

Fig. 1. Lineweaver-Burke plot for *Spirogyra rhizobrachialis*

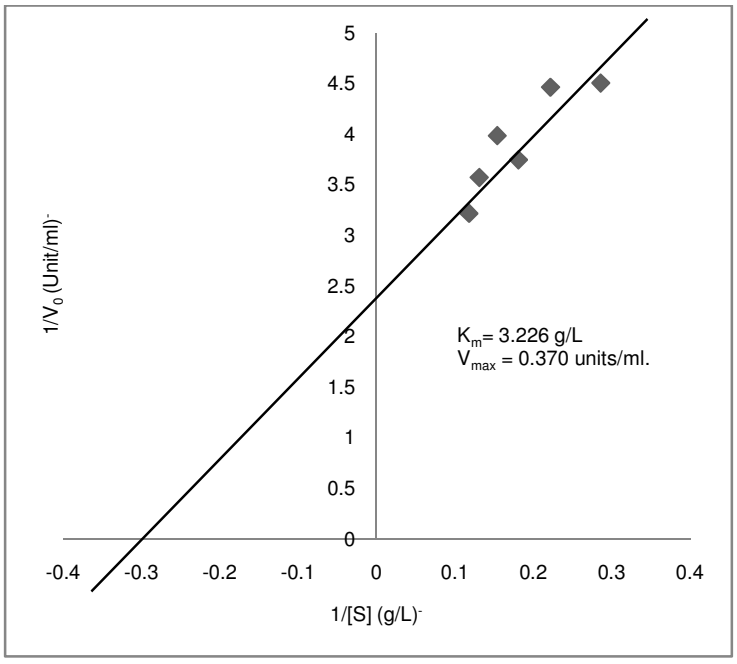

Fig. 2. Lineweaver-Burke plot for *Merismopedia elegans*

The effect of temperature on proteases activity from *S. rhizobrachialis*, *M. elegans* and *Synedra spp.* are presented in Figs. 4, 5 and 6 respectively and their respective optimum temperatures were revealed. Protease activity from *S. rhizobrachialis* was found to be optimum at 47℃, *M. elegans* and *Synedra spp.* to be 45ºC and 50℃ respectively. The optimum temperatures for the three species are temperatures the respective proteins assume the conformation best suited for catalysis. These values are within the range previously reported for optimum protease activity for protease producing bacteria from upper respiratory tract of wild chicken at 45℃ [21], *Aspergillus niger* at 45℃ [19], *Bacillus subtilis* at 55℃ [22]. An optimum temperature of 60℃ was however reported for serine protease from *Pseudomonas aeruginosa* [23].

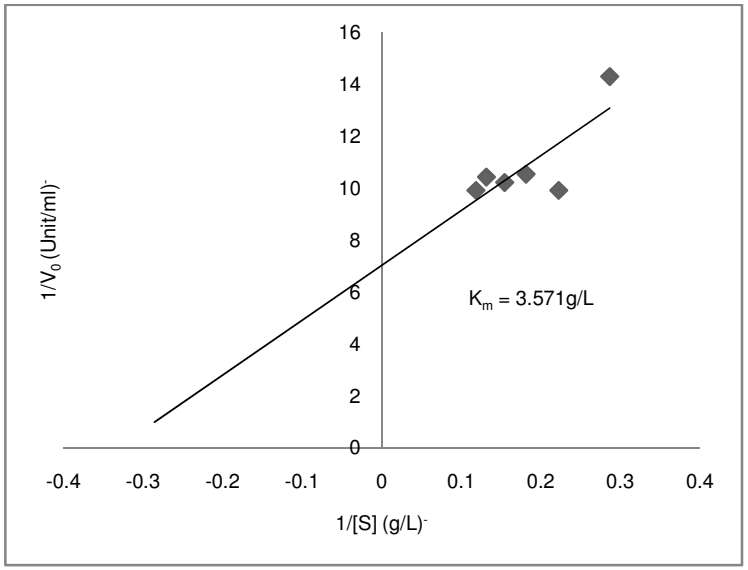

Fig. 3. Lineweaver-Burke plot for *Synedra spp*

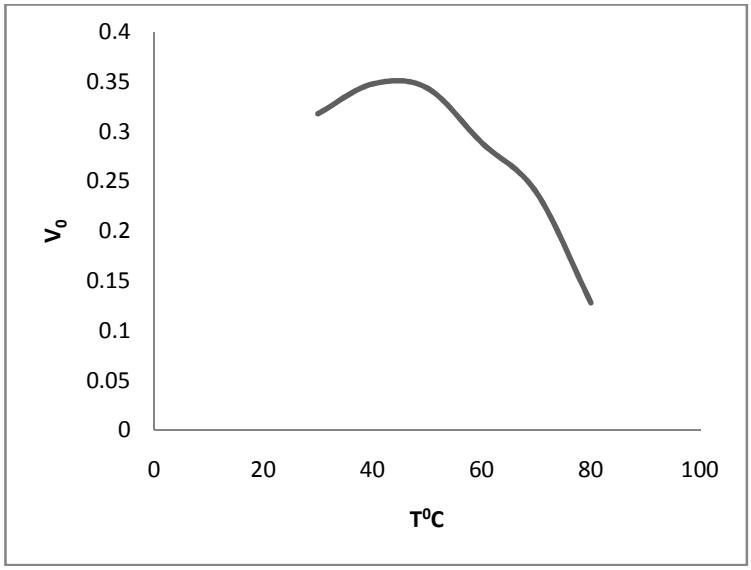

Fig. 4. Effect of temperature on protease activity from *S. rhizobrachialis*

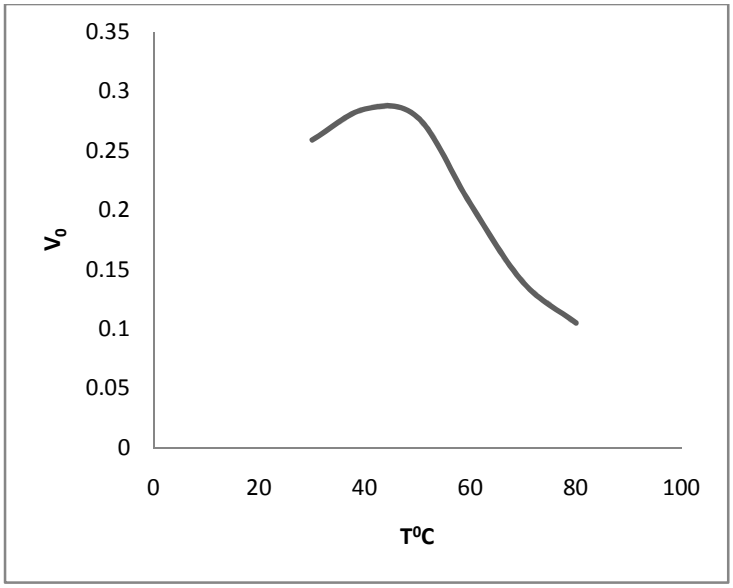

Fig. 5. Effect of temperature on protease activity from *M. elegans*

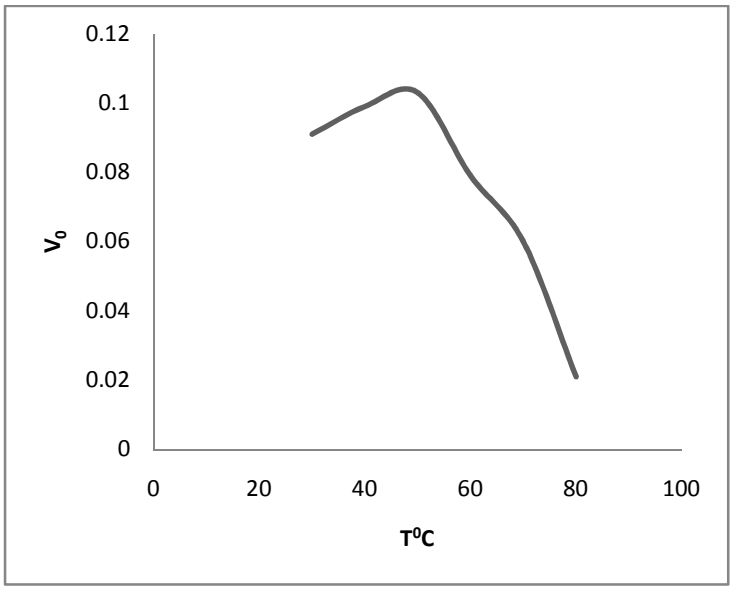

Fig. 6. Effect of temperature on protease activity from *Synedra species*

The effect of pH on the protease activity for *S. rhizobrachialis*, *M. elegans* and *Synedra spp* are presented in Figs. 7, 8 and 9 respectively. Protease from *S. rhizobrachialis* had an optimum pH of 6. Proteases from *M. elegans* and *Synedra spp* had optimum pH 8.This indicates that two forms of proteases i.e acid and alkaline proteases were studied. An optimum pH of 6 for protease activity from *S. rhizobrachialis* is an indication that the most probable amino acids within its active site are those with an acidic side chain. An optimum pH of 8 for both *M. elegans* and *Synedra spp.* protease activities is an indication that the most probable amino acids at their active sites are alkaline in nature. Earlier studies reported an optimum protease activity for *Aspergillus niger* at pH 8.5 [19], *Bacillus subtilis* at pH 8.5 [22], *Pseudomonas aeruginosa* at pH 7.1 [23] and protease producing bacteria from upper respiratory tract of wild chicken at pH 9 [21].

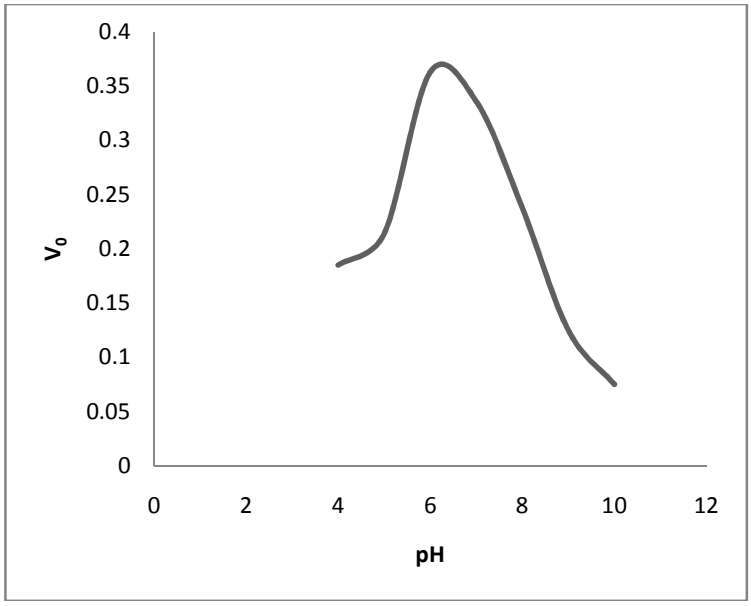

Fig. 7. Effect of pH on activity of protease from *S. rhizobrachialis*

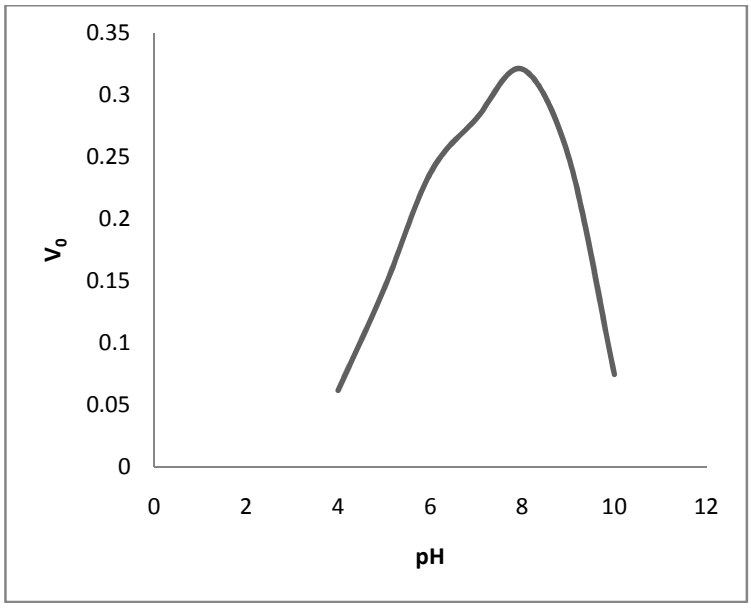

Fig. 8. Effect of pH on activity of protease from *M. elegans*

The effect of some divalent cations on the protease activity for *S. rhizobrachialis*, *M. elegans* and *Synedra spp* is presented in Fig. 10. In all three *Algae* species, Ca^{2+} and Mg^{2+} acted as activators of proteases, while Hg^{2+} is inhibitor of the protease activities. Muthulakshmi et al. [20] reported and Abou-Elela et al. [24] reported Ca^{2+} and Mg^{2+} to inhibit protease activity from *Aspergillus flavus* and *Bacillus cereus respectively*. Manganese served also as an activator for the protease in all three species but *Synedra spp.* where it inhibited the activity. In all three *Algae* species, Hg^{2+} was found to inhibit the enzyme. Protease from *Bacillus cereus* was reported to be inhibited by Mn^{2+} and Hg^{2+} [24-25].

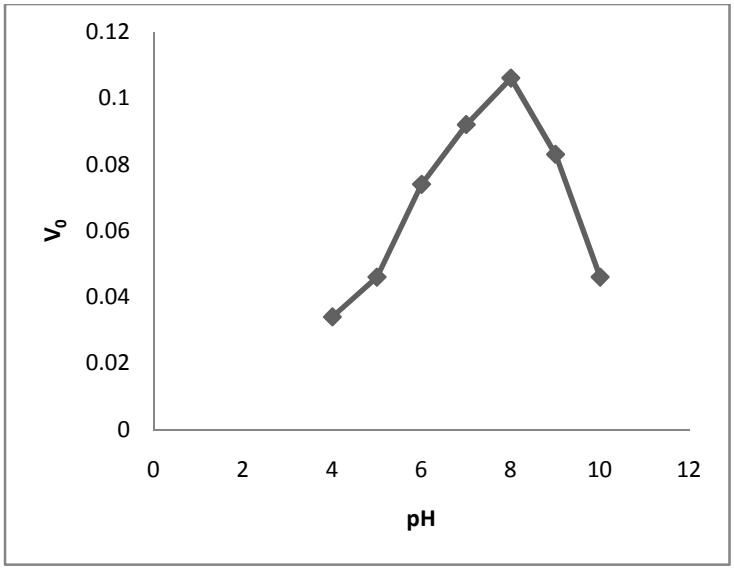

Fig. 9. Effect of pH on activity of protease from for *Synedra spp*

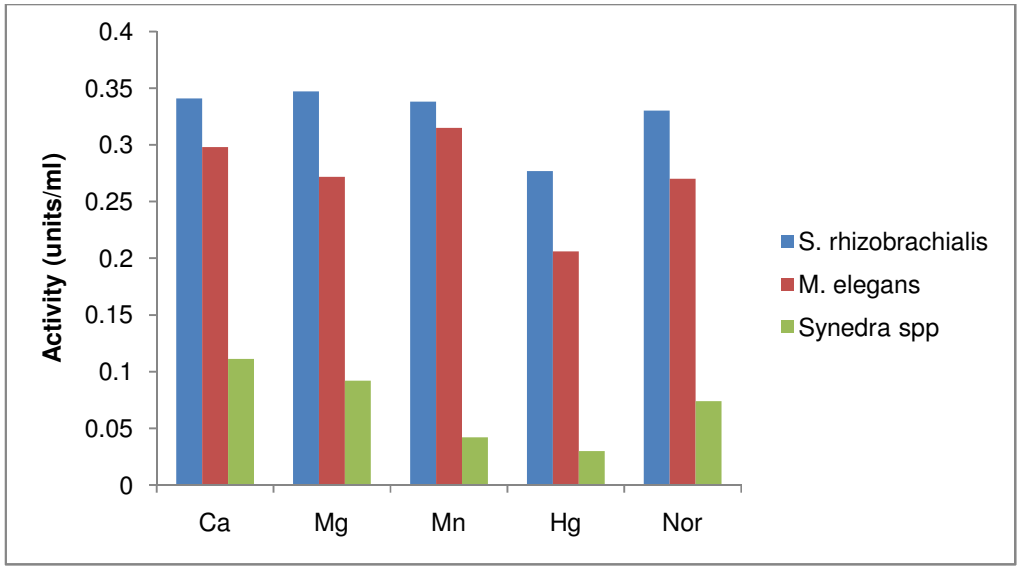

Fig. 10. Effect of metal ions on the activity of proteases from *S. rhizobrachialis, M. elegans and Synedrs spp.*

4. CONCLUSION

Protease was produced by *Spirogyra rhizobrachialis*, *Merismopedia elegans* and *Synedra species*. However, protease from *Spirogyra rhizobrachialis* was found to have high affinity and better specificity and thus could serve as a better industrial source for protease amongst the three species studied.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rawlings ND, Barrett AJ, Bateman A. Asparagine peptide lyases: A seventh catalytic type of proteolytic enzymes. Journal of Biological Chemistry. 2011;286:38321-38328.
2. Chouyyok W, Wongmongkol N, Siwarungson N, Prichnont S. Extraction of alkaline protease using an aqueous two-phase system from cell free *Bacillus subtilis* TISTR 25 fermentation broth. Process Biochemistry. 2005;40:3514–3518.
3. Barindra S, Debashish G, Malay S, Joydeep M. Purification and characterization of a salt, solvent, detergent and bleach tolerant protease from a new gamma *Proteobacterium* isolated from the marine environment of the Sundarbans. Process Biochemistry. 2006;41:208–215.
4. Paranthaman R, Alagusundaram K, Indhumathi J. Production of protease from rice mill wastes by *Aspergillus niger* in solid state fermentation. World Journal of Agricultural Science. 2009;5(3):308-312.
5. Stoner MR, Dale DA, Gualfetti PJ, Becker T, Manning MC, Carpenter JF, Randolph TW. Protease autolysis in heavy-duty liquid detergent formulations: Effects of thermodynamic stabilizers and protease inhibitors. Enzyme and Microbial Technology. 2004;34:114-125.
6. Lund H, Kaasgaard S, Skagerlind P, Jorgensen L, Jorgensen C, Van De Weert M. Correlation between enzyme activity and stability of a protease, an alpha-amylase and a lipase in a simplified liquid laundry detergent system, determined by differential scanning calorimetry. Journal of Surfactants and Detergents. 2011;15:9-21.
7. Daugherty AB, Muthu P, Lutz S. Novelprotease inhibitors via computational redesign of subtilisin BPN' propeptide. Biochemistry. 2012;51(41):8247-8255.
8. Smoum R, Rubinstein A, Dembitsky VM, Srebnik M. Boron containing compounds as protease inhibitors. Chemical Review. 2012;112:4156-4220.
9. Klenk HD, Garten W. Host cell proteases controlling virus pathogenicity. Trendsin Microbiology. 1994;2:39.
10. Katherine MB, Alfred LG, Matthew B, Douglas H, Johanna AJ. Inhibition of cysteine cathepsin protease activity enhances chemotherapy regimens by decreasing tumor growth and invasiveness in a mouse model of multistage cancer. Cancer Research. 2007;67:7378-7385.
11. Madan M, Dhillon S, Singh R. Production of alkaline protease by a UV mutant of *Bacillus polymyxa*. Ind J Microbiol. 2002;42:155-159.
12. Sandhya C, Sumantha A, Szakacs G, Pandey A. Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. Process Biochemistry. 2005;40:2689–2694.
13. Haq IU, Mukhtar H, Umber H. Production of protease by *Penicilliumchry sogenum* through optimization of environmental conditions. Journal of Agricultural Social Science. 2006;2(1):23–25.
14. Maugh TH. A renewed interest in immobilised enzymes. Science. 1984;223:474-476.
15. Lowry B, Krishna S, DeviK L. Optimization of thermos table alkaline protease production from species of *Bacillus* using rice bran. African Journal of Biotechnology. 2005;4(7):724-726.
16. Kabli SA. Purification and characterization of protopectinase produced by *Kluyveromyces marxianus*. *Journal of King* Abdulaziz University Science. 2007;19:139-153.
17. Bob M. Hypsibius dujardini collection notes and culture protocols; 2007.
18. Carrie C. Sigma's non-specific protease activity assay - casein as a substrate. Journal of Visualize Experiment. 2008;19:899.
19. Devi K, Banu R, Gnanaprabhal GR, Pradeep BV, Palaniswamy M. Purification, characterization of alkaline protease enzyme from native isolate *Aspergillus niger* and its compatibility with commercial detergents. Indian Journal of science and technology. 2008;1:7.
20. Muthulakshmi C, Gomathi D, Kumar DG, Ravikumar G, Kalaiselvi M, Uma C. Production, Purification and Characterization of Protease by *Aspergillus flavus* under solid state

fermentation. Jordan Journal of Biological Sciences. 2011;4(3):137-148.
21. Raut S, Sen SK, Kabir NA, Satpathy S, Raut S. Isolation and characterization of protease producing bacteria from upper respiratory tract of wild chicken. Bio information. 2012;8(7):326-330.
22. Ikram H, Mukhtar H. Kinetic and thermal characterisation of alkaline protease produced by a new alkalophilic isolate of *Bacillus subtilis*. Journal of chemical society of Pakistan. 2008;30.
23. Izrael-Živković L, Gojgić-Cvijović G, KaradžićI. Isolation and partial characterization of protease from *Pseudomonas aeruginosa* ATCC 27853. Journal of Serbian Chemical Society. 2010;75(8):1041–1052.
24. Abou-Elela GM, Ibrahim HAH, Hassan SW, Abd-Elnaby H, El-Touky NMK. Alkaline protease production by alkaliphilic marine bacteriai solated from Marsa-Matrouh (Egypt) with special emphasis on *Bacillus cereus* purified protease. African Journal of Biotechnology. 2011;10(22):4631-4642.
25. Kavitha B, Thankamani V. Purification and characterization of protease enzyme from *Bacillus cereus*. Research Journal of Biotechnology. 2012;7(3):88-91.

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