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Purification and Characterization of a Protease from Green Seeded Chickpea (*Cicer arientum*)

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Abstract

Background: Proteases are ubiquitous in nature and are found to play a major role in biological functions like digestion of food, control of blood clotting, apoptosis and interaction of signalling receptors in animals.

Method: The protease was purified by soaking of sample, homogenization, ammonium sulphate precipitation and Ion-exchange chromatography on DEAE-cellulose column. Activity of protease was determined using Nα-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as chromogenic substrate. The biochemical characterization was done for temperature stability, pH profile and effect of various inhibitors on enzymatic activity.

Results: We have purified a trypsin like protease of ~23 kDa from Cicer arientum, green chickpea seeds. The protease shows appreciable stability in wide range of pH and temperature.

Conclusion: To the best of our knowledge, it is first report on purification of protease with trypsin-like properties, from this source.

Keywords: Trypsin; Cicer arientum; Protease; Protein purification; BAPNA

Introduction

Proteases or proteinases are the enzymes that perform proteolysis i.e. digestion of proteins [1]. They are involved extensively in a large number of biological activities and hence are a key enzyme for survival and maintenance of the organism [2-3]. In the course of evolution, proteases have adapted well with wide range of conditions (including variations in pH, reductive environment etc.) which make them suitable for complex organisms. They are classified on the basis of the use of different catalytic mechanisms for substrate hydrolysis [4]. Their mechanism of action classifies them as serine, cysteine or threonine proteases or as aspartic, metallo and glutamic protease. Proteases specifically cleave protein substrates either from the N or C termini (aminopeptidases and carboxypeptidases, respectively) or/and anywhere except the ends of the molecule (endopeptidases). By cleaving proteins, proteases are involved in the control of a large number of key physiological processes such as cell-cycle progression, DNA replication, cell proliferation and cell death, tissue remodelling, coagulation (homeostasis), wound healing and the immune response [4].

Chickpea (*Cicer arientum*) constitutes an important source of dietary protein and carbohydrate and ranks third among pulses [5]. Chickpea is consumed fashionably in large amounts in various forms like sprouts, seeds and salads [6]. Chickpea has been found in reducing the risks of many diseases like cancer, diabetes, obesity, skin and blood disorders, liver and spleen diseases etc [7-11].

Chickpea has served as a source of Protease Inhibitor. A chickpea Kunitz-type inhibitor showing activity against trypsin and *Helicoverpa armigera* gut proteases (HGP) has previously been reported by Srinivasan et al. [12]. Also, isoinhibitors of Trypsin and Chymotrypsin in Chick Peas (*Cicer arientum* L.) were purified by Makonnen et al. [13]. Sharma et al. have also purified trypsin inhibitor from chickpea seeds (CPTI) and reported it as member of Kunitz soybean trypsin inhibitor (KSTI) family having a molecular mass of about 18-20 kDa and containing two disulfide bridges and a single reactive site for trypsin [14]. Also, Smirnoff et al. purified protease inhibitor from chickpea and reported its activity against both trypsin and chymotrypsin proteases [15]. Studies have revealed the purification of protease from various sources of plants and animals [16-19] but presence and isolation of protease from *Cicer arientum* 'Green chickpea' have not been reported yet. Therefore, we have undertaken to purify and study the protease for some of its physical and chemical properties. Its stability under various conditions as well as its specificity towards different proteolytic inhibitors is also described. The study revealed chickpea as a source of novel protease. This study may be helpful in understanding functions of proteases as well as in exploring new sources of proteases. Also such studies, to some extent, can contribute to the understanding of significant role of proteases in industrial uses.

Materials and Methods

Chemical and reagents

C. arientum, commonly known as *'hari matar'* was purchased from the local market. Protease activity assay and SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) chemicals; trypsin (porcine pancreatic trypsin), BAPNA, acrylamide, bis-acrylamide, Tetramethylethylenediamine (TEMED), ammonium persulphate; were obtained from Sigma-Aldrich. All other chemicals used were of analytical grade.

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Purification of protease from *Cicer arientum*

Fresh *Cicer arientum* (Green chickpea seeds) were homogenized in a blender using 20mM Tris HCl buffer with 2 mM $CaCl_2$ (pH 8.2). The crude extract was filtered through muslin cloth to remove the coarse residual matter. It was then centrifuged at 8000 rpm for 45 min at 4°C. The supernatant was defatted using glacial acetic acid to drop pH to 4 and kept on stirring for 4 hrs. The defatted homogenate was collected from supernatant after centrifugation at 8000 rpm for 45 min at 4°C. It was then followed by maintaining pH to 7 by liquor ammonia. The supernatant was raised to 30-50% saturation by adding ammonium sulphate. After overnight stirring at 4°C, it was again centrifuged at 8000 rpm for 45 min at 4°C to precipitate the proteins salted out by ammonium sulphate. The protein pellet obtained was dissolved in minimal amount of 20 mM Tris- 2 mM $CaCl_2$ buffer (pH 8.2) and dialyzed against the same buffer extensively to remove ammonium sulphate. The dialyzed sample was applied to a DEAE-cellulose column for further purification. The unbound proteins were washed with 20 mM Tris- 2 mM $CaCl_2$ buffer (pH 8.2) and the desired protein was eluted at 0.2 M NaCl in the same buffer.

Protein estimation

Measurement of protein concentration was done by the method of Lowry et al. [20] using bovine serum albumin (1 mg/ml) as a standard and the concentration was expressed in milligram per milliliter (mg/ml).

SDS PAGE analysis

SDS-PAGE was performed on 12% polyacrylamide slab gel under reducing conditions using the method of Laemmli [21]. The molecular weight was estimated by comparing the sample protein band with standard trypsin.

Determination of protease activity

Protease activity was measured according to the method of Erlanger et al. [22] using BAPNA as a substrate. Samples of each purification step (50 µl) were added to 50 µl of 20 mM Tris-HCl buffer with 2 mM $CaCl_2$ (pH 8.2). To initiate the reaction, 800 µl of BAPNA (1.5 mM in 20% glycerol) was added and mixed thoroughly. After incubation for 10 min at 25°C, 100 µl of 30% acetic acid (v/v) was added to terminate the reaction. The absorbance of reaction mixture was read at 410 nm.

Activity of protease was calculated by formula:

$$\text{Activity U/1} = \frac{\text{Absorbance x Volume of reaction mixture (ml) x } 10^6}{\text{molar extinction coeff. of BAPNA x Sample volume (ml)}} \times 1000$$

where, molar extinction coeff. of BAPNA is 8270 $cm^{-1}M^{-1}$

Effect of temperature

The thermal stability of the Protease was done in 20 mM Tris-HCl buffer (pH 8.2) by incubating the sample at various temperatures, i.e. 20°C, 30°C, 37°C, 50°C and 60°C for 60 min. The experiment was performed in duplicates. The residual activity of the samples was checked at 410 nm using Spectrophotometer (Schimadzu, Japan) with 15 min. interval using BAPNA as substrate. The activity of protease without incubation was calculated as per the formula mentioned above. It was considered as 100%. The mean value of the duplicate set was considered as test results. Percentage residual activity was calculated by:

Residual activity(%) = $Abs_{test}/Abs_{control}$ x100

Effect of pH

The effect of pH on the activity of Protease was checked by incubating it at the desired pH (2.0–10.0) for 60 min at 25°C and then assaying for residual enzyme activity as mentioned previously (in duplicates). 10 mM solutions of the following buffers were used to get the desired pH: Gly-HCl (pH 3.0–4.0), sodium acetate (5.0–6.0), Tris-HCl (pH 7.0–8.0) and Gly- NaOH (pH 9.0–10.0). The mean value of the duplicate set was considered as test results. Percentage residual activity was calculated by:

Residual activity(%) = $Abs_{test}/Abs_{control}$ x100

Effect of inhibitors

Inhibition was measured according to the method of Garcıa-Carreno and Haard [23] and Garcıa-Carreno [24]. Enzyme extracts were incubated with different specific protease inhibitors, such as the serine-protease inhibitors; Phenyl-methyl-sulphonyl-fluoride (PMSF), Lima Bean Trypsin Inhibitor (LBTI purchase from Sigma-Aldrich, Lot# SLBB6142V) and *Cajanus cajan* Trypsin Inhibitor (CCTI purified in laboratory) and the metalloprotease deactivator Ethylenediamine tetraacetic acid (EDTA). A mixture of 100 µl inhibitor solution and 100µl enzyme extract were incubated for 60 min at 25°C, and then 750 µl substrate solution (1.5M BAPNA in 20% glycerol) was added. After incubation for 10 min at 25°C, 50 µl of 30% acetic acid (v/v) was added to terminate the reaction and residual activity was measured. The experiment was performed in duplicates and mean value was considered to calculate percentage residual activity, considering activity in the absence of inhibitor as 100%.

Results

Purification of protease from green chickpea

The 30-50% ammonium sulphate fraction, which showed maximum Protease activity, was initially loaded onto a DEAE-cellulose column. The protein was eluted with same buffer containing 0.2M NaCl and the absorbance was recorded at 280 nm (Figure 1). Fractions which showed maximum absorbance at 280 nm were checked for proteolytic activity. Fractions showing maximum activity were pooled and dialyzed against the same buffer to remove NaCl. This preparation was used for all further studies.

Determination of amount, molecular mass and purity

12% SDS polyacrylamide gel electrophoresis of the eluted Protease yielded a prominent band at approximately 23 KDa (lane 3) as

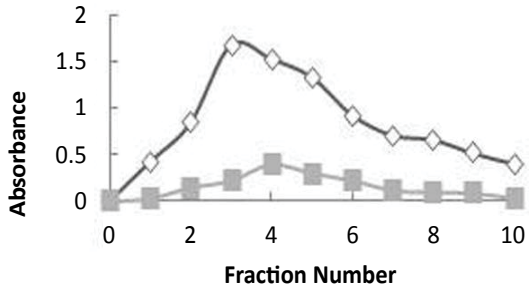

Figure 1: The dialyzed fraction obtained after (30-50%) ammonium sulfate precipitation was further purified by ion-exchange chromatography on DEAE-cellulose column equilibrated by 20 mM TrisHCl buffer with 2 mM $CaCl_2$ (pH 8.2). Elution was performed in same buffer containing 0.2M NaCl and collected in 2 ml fractions. Each fraction was assayed for trypsin activity and protein concentration by determining absorbance at 410 nm (•) and 280 nm (◊) respectively.

compared to the standard Protease from porcine pancreas of mol wt. 23.8 KDa (lane 2). The molecular marker was used to determine the molecular weight (lane 1) (Figure 2).

Determination of protease activity from green chickpea

The amount and concentration of Protease was determined by Lowry et al. [25]. The activity assay of Protease was performed using BAPNA as per the protocol mentioned above. As given in table 1, the method yielded good amount of protease (i.e. 70% of the total) and was 10-fold purified as compared to the crude extract.

Effect of temperature

The protein isolated showed thermo-stability between the range 4°C to 37°C. The activity drops significantly at 50°C and the protein is denatured at 60°C (Figure 3).

Effect of pH

Chickpea protease was quite stable in the neutral to mild alkaline pH range. It showed maximum activity at around pH 8 which dropped to approximately 50% at pH around 5-6 (Figure 4). In general, protein stability is related to net charge at a particular pH.

Effect of inhibitors

Inhibition of the isolated protease was checked by different inhibitors. Activity was inhibited to 100% by the serine-protease inhibitor PMSF, but was least affected by the metallo-protease inactivator EDTA (Table 2). This protease was significantly inhibited by protein protease inhibitors, CCTI and LBTI showing 20% and 6% residual activity after inhibition. These results establish that the isolated enzyme specifically is a trypsin-like serine protease.

Discussion

Proteases have a wide range of applications including their role as components of laundry detergents to remove proteinaceous stains from clothes [26] and in textile industry, to remove the stiffness and improving lustre of raw silk [27]. They are also key components in biopharmaceutical products such as contact-lens enzyme cleaners and enzymic deriders [28]. Proteases and their inhibitors may also be used as early diagnostics and prognostic cancer biomarkers to enable selection of the appropriate therapy [4]. The proteolytic enzymes have also proved their efficacy in the successful local management of skin ulcerations by the efficient removal of the necrotic material [29]. Therefore, analysis of the activity of the proteases in vivo is currently one of the hottest areas of protein research [29,30]. Owing to these wide range of applications, we have tried to purify protease and these results confirm that the isolated enzyme is a serine protease. The specificity of chickpea protease towards BAPNA was similar to pattern of protease from other sources like *Monterey sardine* [16].

Chickpea protease showed maximum activity at pH around 8 which dropped to approximately 50% at pH between 5-6 like sardine protease which was stable in the pH range from 7 to 8 [16-18]. Here, chickpea protease showed similar pattern like fish protease which are unstable at acid or extremely alkaline pH [25]. In case of thermo-stability, chickpea protease has characteristics similar to those of purified from fish sources, i.e. it is very stable at temperatures lower than 45°C and increasingly inhibited above 55°C [18,25,31-33].

It has been established that extracellular proteases that act on matrix proteins play a crucial role in tumor invasion and metastasis [34]. Trypsin also shows a significant proteolytic activity towards a large number of extracellular matrix proteins including laminin and fibronectin [35]. It acts as inhibitor for a variety of MMPs and serine proteases [36-37]. Therefore, the protease thus purified can play a vital role in industries as well as in the field of medicine and health.

Conclusion

We have successfully isolated protease from the seed of *Cicer*

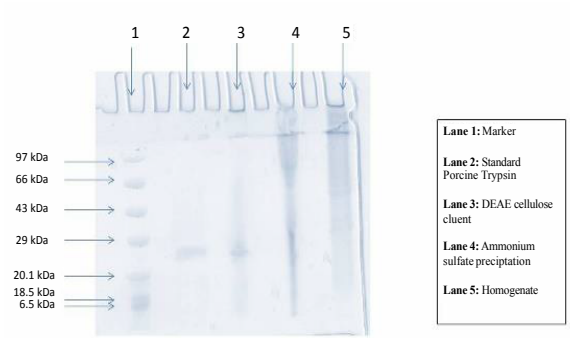

Figure 2: Electrophoretogram with standard marker (lane 1) with standard trypsin from porcine pancreas (lane 2) compared to purified protease from *C. arientum* (lane 3). Lanes 4 and 5 are 30-50% ammonium sulphate fraction and crude homogenate respectively.

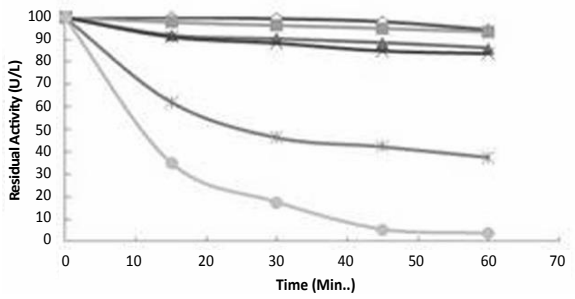

Figure 3: Effect of temperature on enzymatic activity Chickpea protease was submitted to various temperatures i.e. 4°C (◇), 20°C (■), 30°C (▲), 37°C(x), 50°C (∗) and 60°C (●). Residual tryptic activity was measured at the interval of 15 min. after bringing back to room temperature and using BAPNA as a substrate and represented as the % of the residual activity of untreated Trypsin. Each point is the mean of two independent assays.

Step	Activity (U/ml)	Protein Conc. (mg/ml)	Amount (ml)	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Fold Purification	Yield (%)
Homogenate	13397.82	4.88	100	488.5	1339782.35	2742.64	1	100
30-50% $(NH_4)_2SO_4$ precipitate	56469.16	2.26	20	45.2	1129383.31	24986.35	9.1	84.3
DEAE-cellulose Eluent	62716.64	2.3	15	34.87	940749.69	26978.76	9.8	70.2

Table 1: Details of protein, its concentration, activity and purity at each step of purification.

Inhibitors	Concentration (mg/ml)	Residual enzymatic activity (%) of Protease
PMSF	2.5	100
LBTI	1	6
CCTI	1	20
EDTA	0.25	85

Table 2: Effect of Inhibitors on residual enzymatic activity.

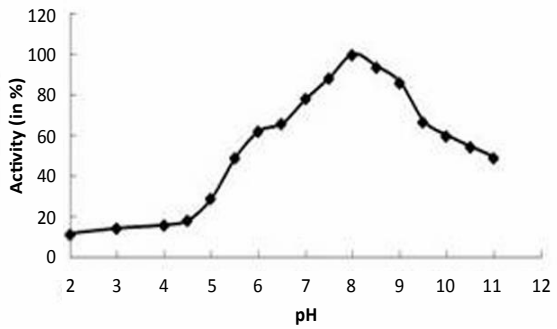

Figure 4: Chickpea protease was incubated with different pH buffers for 60 min at 25ºC. Residual tryptic activity was measured using BAPNA as a substrate and represented as the % of the residual activity as compared to untreated Trypsin (◆). Each point is the mean of two independent assays.

arientum (Green chickpea) in limited steps with high yield. The seeds were soaked overnight in Tris buffer (pH 8.2) containing 20 mM Tris- 2 mM $CaCl_2$ buffer followed by homogenization and centrifugation. The protein was precipitated in 30-50% fraction of ammonium sulphate precipitation and dialysis. The dialyzed sample was loaded on DEAE-cellulose column for Ion-exchange chromatography and the protease was eluted in 0.2M NaCl Elution buffer. The fractions were observed and the peak was characterized for concentration, molecular mass using SDS PAGE and activity using BAPNA. Based on activity for the specific substrate BAPNA and its susceptibility to inhibitors like CCTI and LBTI, the isolated enzyme was concluded to be a protease. And hence, we found out that our purification procedure is rapid, efficient, easy, less time consuming and produces protease with high purity. Also, characterization of the isolated protease showed an interesting outlook from the technological perspective, especially maximum activity at pH 8.0, high neutral pH activity at optimal activity temperature at 50°C. These characteristics suggest that the enzyme shows trypsin-like property which could be an important biotechnological tool for tissue culture, proteomics, cancer research and food industries.

Authors' Contribution

TNS. designed the study and performed the experimental work and data analyses. PS. has been helpful throughout. SF. supervised the experimental work. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that there are no conflict of interest involved.

References

1. Habib H, Khalid MF (2007) Plant protease inhibitors: a defense strategy in plants. Biotechnology and Mol Biol Rev 2: 68-85.
2. Joanitti GA, Freitas SM, Silva LP (2006) Proteinaceous Protease Inhibitors: Structural Features and Multiple Functional Faces. Curr Enz Inhib 2: 199-217.
3. Neurath H (1989) Proteolytic processing and physiological regulation. Trends Biochem Sci 14: 268-271.
4. Turk B (2006) Targeting proteases: successes, failures and future prospects. Nat Rev 5: 785-799.
5. Duke JA (1981) Handbook of legumes of world economic importance. Starch 34: 52-57.
6. Panlasigui LN, Panlilio LM, Madrid JC (1995) Glycaemic response in normal subjects to five different legumes commonly used in the Philippines. Int J Food Sci Nutr 46: 155-160.
7. Prathapan A, Fahad K, Thomas BK, Philip RM, Raghu KG (2011) Effect of sprouting on antioxidant and inhibitory potential of two varieties of Bengal gram (*Cicer arietinum* L) against key enzymes linked to type-2 diabetes. Int J Food Sci Nutr 62: 234-238.
8. Kumar S, Kapoor V, Gill K, Singh K, Xess I, et al. (2014) Antifungal and antiproliferative protein from *Cicer arietinum*: a bioactive compound against emerging pathogens. Bio Med Res Int 387203: 1-15.
9. Tiwari AK, Sahana C, Zehra A, Madhusudana K, Kumar DA, et al. (2013) Mitigation of starch-induced postprandial glycemic spikes in rats by antioxidants-rich extract of *Cicer arietinum* Linn. seeds and sprouts. J Pharm Bioall Sci 5: 270-276.
10. Leterme P (2002) Recommendations by health organizations for pulse consumption. Br J Nutr 88: 239-242.
11. Qiaoying LV, Yang Y, Zhao Y, Gu D, He D, et al. (2009) Comparative Study on Separation and Purification of Isoflavones from the Seeds and Sprouts of Chickpea by HSCCC. J Liq Chrom Relat Tech 32: 2879-2892.
12. Srinivasan A, Chougule NP, Giri AP, Gatehouse JA, Gupta VS (2005) Podborer (*Helicoverpa armigera Hübn*) does not show specific adaptations in gut proteinases to dietary *Cicer arietinum* Kunitz proteinase inhibitor. J Insect Physiol 51: 1268-1276.
13. Makonnen BM, Porath J, Sundberg L (1975) The Trypsin and Chymotrypsin Inhibitors in Chick Peas (*Cicer arietinum* L.). Eur J Biochem 60: 247-258.
14. Sharma U, Suresh CG (2011) Purification, crystallization and X-ray characterization of a Kunitz-type trypsin inhibitor protein from the seeds of chickpea (*Cicer arietinum*). Acta Crystallogr Sect F Struct Biol Cryst Commun 67: 714-717.
15. Smirnoff P, Khalef S, Birk Y, Applebaum SW (1976) A Trypsin and Chymotrypsin Inhibitor from Chick Peas (*Cicer arietinum*). Biochem J 157: 745-751.
16. Castillo-Yanez FJ, Pacheco-Aguilar R, Garcia-Carreno FL, Navarrete-Del, Toro MA (2005) Isolation and characterization of trypsin from pyloric caeca of *Monterey sardine Sardinops sagax caerulea*. Comp Biochem Physiol B Biochem Mol Biol 140: 91-98.
17. Singh SJ, Singh LR, Devi SK, Singh SS, Devi CB, et al. (2015) Purification and Characterization of a Thermostable Caseinolytic Serine Protease from the Latex of *Euphorbia heterophylla* L. Protein Pept Lett 22: 828-835.
18. Ishwarya S, Sangeetha R. (2013) A new serine protease from the leaves of *Thespesia populnea*. Prep Biochem Biotechnol 43: 95-107.
19. Ranjbar M, Zibaee A, Sendi JJ (2014) A trypsin-like proteinase in the midgut of *Ectomyelois ceratoniae Zeller* (Lepidoptera: Pyralidae) purification, characterization, and host plant inhibitors. Arch Insect Biochem Physiol 85: 1-12.
20. Lowry OH, Rosenbrough NJ, Farr AL, Randal RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265-275.
21. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature 277: 680–685.
22. Erlanger BF, Kokowsky N, Cpenhen W (1961) The preparation and properties of two new chromogenic substrates of trypsin. Arch Biochem Biophys 95: 271-278.
23. Garcia-Carreno FL, Haard NF (1993) Characterization of proteinase classes in langostilla (*Pleuroncodes planipes*) and crayfish (*Pacifastacus astacus*) extracts. J Food Biochem 17: 97-113.
24. Garcia-Carreno FL (1996) Proteinase inhibitors. Trends in Food Science and Technology 7: 197-203.

25. Haq SK, Khan RH (2003) Characterization of a proteinase inhibitor from *Cajanus cajan* (L.). J Protein Chem 22: 543-554.

26. Banerjee UC, Sani RK, Azmi W, Soni R (1999) Thermostable alkaline protease from *Bacillus brevis* and its characterization as a laundry detergent additive. Process Biochem 35: 213-219.

27. Najafi MF, Deobagkar D, Deobagkar D (2005) Potential application of protease isolated from *Pseudomonas aeruginosa* PD100. Electron J Biotechnol 8: 197-203.

28. Neefjes J, Dantuma NP (2004) Fluorescent probes for proteolysis: tools for drug discovery. Nat Rev Drug Discov 3: 58-69.

29. Sjpdahl J, Emmer A, Vincent J, Roeraade J (2002) Characterization of proteinases from Antarctic krill (*Euphausia superba*). Protein Express Purif 26: 153-161.

30. Baruch A, Jeffery DA, Bogyo M (2004) Enzyme activity- it's all about image. Trends Cell Biol 14: 29-35.

31. Murakami K, Noda M (1981) Studies on proteinases from the digestive organs of sardine-purification and characterization of three alkaline proteinases from the pyloric caeca. Biochim Biophys Acta 658: 17-26.

32. Heu MS, Kim HR, Pyeun JH (1995) Comparison of trypsin and chymotrypsin from the viscera of anchovy (*Engraulis japonica*). Comp Biochem Physiol B Biochem Mol Biol 112: 557-567.

33. Martinez A, Olsen RL, Serra J (1988) Purification and characterization of trypsin like enzymes from the digestive tract of anchovy (*Engraulis encrasicholus*). Comp Biochem Physiol B Biochem Mol Biol 91: 677-684.

34. Stetler-Stevenson WG, Aznavoorian S, Liotta LA (1993) Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Dev Biol 9: 541-573.

35. Koshikawa N, Yasumitsu H, Umeda M, Miyazaki K (1992) Multiple secretion of matrix serine proteinases by human gastric carcinoma cell lines. Cancer Res 52: 5046-5053.

36. Okada Y, Gonoji Y, Naka K, Tomita K, Nakanishi I, et al. (1992) Matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase) from HT 1080 human fibrosarcoma cells. Purification and activation of the precursor and enzymic properties. J Biol Chem 267: 21712-21719.

37. Imai K, Yokohama Y, Nakanishi, Ohuchi E, Fujii Y, et al. (1995) Matrix metalloproteinase 7 (matrilysin) from human rectal carcinoma cells. Activation of the precursor, interaction with other matrix metalloproteinases and enzymic properties. J Biol Chem 270: 6691-6697.