Biomedical Research and Therapy **2015, 2(9): 354-358**
ISSN 2198-4093
www.bmrat.org

ORIGINAL RESEARCH

COLLAGENOLYTIC ACTIVITY IN TISSUE EXTRACT OF *PARBORLASIA CORRUGATUS* FROM ANTARCTIC REGION

N.G. Raksha*, D.V. Gladun, O.M. Savchuk, L.I. Ostapchenko

Taras Shevchenko National University of Kyiv, ESC 'Institute of Biology', 64/13, Volodymyrska Str., Kyiv 01601, Ukraine
*Corresponding author: natahenkudina@gmail.com

Received: 02 July 2015 / **Accepted**: 01 September 2015 / **Published online**: 10 September 2015

Abstract - Marine organisms have been recognized as rich sources of bioactive compounds with valuable biotechnology potential. Enzymes extracted from marine hydrobionts have gained much attention because of their unique quite specific properties that determined their profound applications in chemical, medical, food industries and molecular biology experiments. In this regard, our work focused on investigation of proteolytic potential of marine hydrobionts. At first, tissue extract of Antarctic hydrobiont *Parborlasia corrugatus* was separated by gel filtration chromatography on a Superdex-75 PG. Further zymography with using gelatin as substrate revealed the presence of clear band that can indicate about active enzymes. It had been shown the presence of collagenolytic activity in all eight fractions obtained after chromatographic separation of tissue extract. Trypsin-like (L-BApNA hydrolyzing) was found only in first fraction. Our results let us assume that *P. corrugatus* can be regarded as potential source of enzymes for practical use.

Keywords: Marine hydrobiont, Gel filtration chromatography, Zymographic technique, Collagenolytic, Trypsin-like activities

INTRODUCTION

!"#$%&'(")*+,%*-%.&"-*/0$*&"-*1'%*0/*+,%*2$0-1#+'*+,%*&#+(3%*#0.20"%"+*0/*4,(#,*('*$%2$%'%"+%-*56*,6-$076+(#*%"86.%'*-%+%$.("%'*+,%*$%7%3&"#%*0/*/("-(")*'"%4*&7+%$9"&+(3%*'01$#%'*0/*,6-$076+(#*%"86.%':*;"/0$+1"&+%76*+,%*("-1'+$(&7*&227(#&+(0"*/0$*/00-<*#0'.%+(#*&"-*2,&$9.&#%1+(#&7*0/*.&..&7(&"*2$0+%&'%'*,&'*$%#%"+76*5%%"*7(.(+%-*56*+,%*01+5$%&=*0/*'0.%*&"(.&7'>*-('%&'%'*0$?&"-*$%7()(01'*+$&-(+(0":*@0$*+,('*$%&'0"'*.&"6*("3%'9+()&+0$'*,&3%*/0#1'%-*+,%($*&++%"+(0"*0"*.&$("%*0$)&"9('.'*(.20$+&"#%*0/*4,(#,*&'*&'01$#%*0/*"%4*'15'+&"#%'*('*)$04(")*!+*',017-*5%*"0+%-*+,&+*+,%($*'1$3(3&7*("*&*3%$6*%A()%"+<*#0.2%+(+(3%*&"-*&))$%''(3%*'1$$01"-(")*#&1'%'*"0+*0"76*#0"'(-%$&57%*'+$1#+1$&7*&"-*/1"#+(0"&7*-(3%$'(+6*0/*.&$("%*5(070)(#&776*&#+(3%*#0.201"-'*51+*&7'0*+,%*2$%'%"#%*0/*%"86.%'*+,&+*.&6*5%*1'%-*("*50+,*5&'(#*$%'%&$#,*&"-*(" -1'+$(&7*&227(#&+(0"'*B@1#,('%*%+*&7:<*CDEEF*G0")=7&"&=&$("<*CDDHI:*G0<*&'*%3071+(0"&$6*2$%#1$9'0$'*0/*2$0+%&'%'*0/*4&$.95700-%-*&"(.&7'<*,6-$07&'%'*0/*#07-94&+%$*.&$("%*("3%$+%5$&+%'*&$%*#,&$&#+%$(8%-*&*"1.5%$*0/*1"(J1%*J1(+%*'2%#(/(#*2$02%$+(%':*K,1'*+,%6*,&3%*'()"(/(#&"+76*,(),%$*+0+&7*2$0+%076+(#*&#+(3(+6*+094&$-'*"&+(3%*2$0+%("*'15'+$&+%'<*5$0&-%$*'15'+$&+%*'2%9#(/(#(+6*&"-*704%$*&#+(3&+(0"*%"%$)6*/0$*#&+&76'('*#0.92&$%-*4(+,*2$0+%&'%'*/$0.*.&..&7(&"*0$*.(#$05(&7*'01$#%':*!"*&--(+(0"*%"86.%'*/$0.*#07-94&+%$*,6-$095(0"+'*&$%*.0$%*&#+(3%*&+*704*+%.2%$&+1$%'*+,&"*+,0'%*/$0.*.&..&7'<*+,%$.02,67(#*0$)&"('.'*&"-*27&"+'*BL%#=*%+*&7:<*CDDMI:*N77*+,('*.&=%'*+,%*2$0'2%#+*0/*+,%($1'%'("*'%3%$&7*("-1'+$(&7*&227(#&+(0"'<*1#,*&'*("*#%$+&(("*/00-*2$0#%''(")*02%$&+(0"'*+,&+*$%J1($%*704*2$0#%''(")*+%.2%$&+1$%':*!"*+%$.'*0/*2$&#+(#&7*227(#&+(0"*%"86.%'*20''%'*#077&)%"076+(#*&#+(3(+6*&$%*3%$6*2%$'2%#+(3%*9*+,%'%*2$0+%&'%'*#&"*5%*&227(%-*("*#,%.(#&7<*.%-(#&7<*/00-*("-1'+$(%'<*5(0+%#,"070)6*&"-*&'*4%77*&'*/0$*.07%#17&$*5(070)6*%A2%$(.%"+':*

MATERIAL AND METHODS

RESULTS AND DISCUSSION

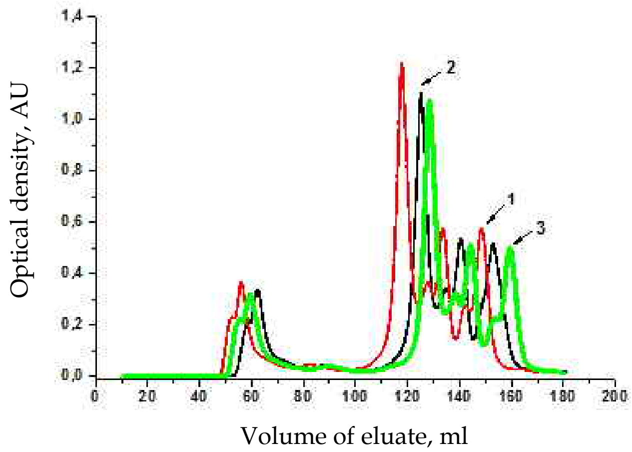

Figure 1. Chromatographic separation of tissue extract of *A. colbecki* at different flow rate: 1 – 1 ml/min; 2 – 0.75 ml/min; 3 – 0.5 ml/min.

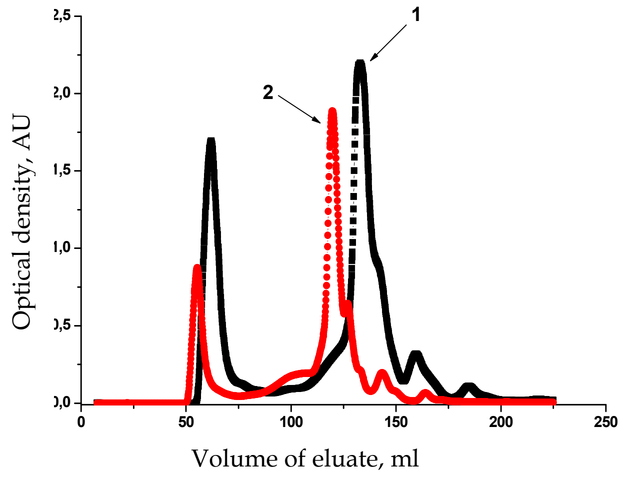

Figure 2.Chromatographic separation of tissue extract of *A. colbecki* using different gel filtration buffer: 1 - 0.05 Mtris-HCl, pH 7.4 with 0.15 M NaCl; 2 – 0.05 Mtris-HCl, pH 7.4.

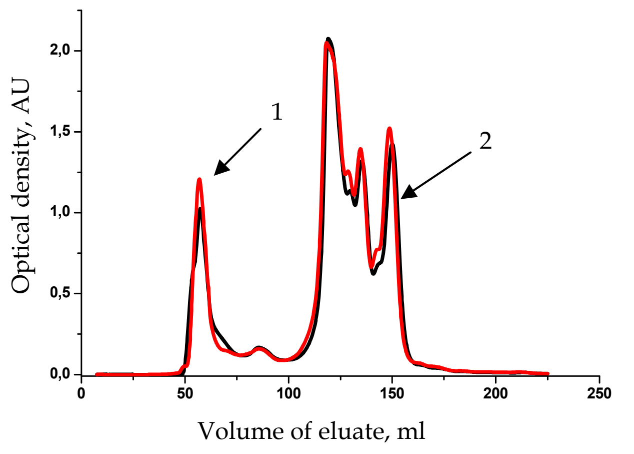

Figure 3. Chromatographic separation of tissue extract of *A. colbecki* using for sample dissolvation: 1 – 0.05 Mtris-HCl, pH 7.4 with 0.5 M NaCl; 2 - 0.05 Mtris-HCl, pH 7.4.

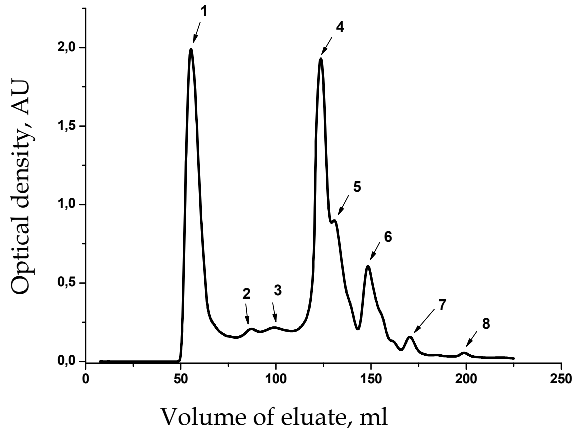

Figure 4.Chromatographic separation of tissue extract of *P. corrugatus*: 1-8 –fraction number.

+0*+, %*3%$+%5$&+%*%"86 . %'*&" -*#&"*5%7O")*+0*+, %*)$012* 0/*'%$("%*2$0+%&'%'*&'* 4%77*&'*+0*+, %*)$012*0/* . %+&77O9 2$0+%&'%':*

N##0$-(")*+0*+, %*7(+%$&+1$%*("*+(''1%'*0/* . &$("%*("3%$+%9 5$&+%'* 4&'* /01"-* &* "1 . 5%$* 0/* , 6-$076+(#* %"86 . %'* 4, (#, *+&=%*2&$+*("*'%3%$&7*/1"-& . %"+&7*2$0#%''%'<*'1#, * &'* %A+$&#%7717&$* . &+$(A* $% . 0-%7(")<* % . 5$60"(#* -%3%79 02 . %"+<*#%77*)$04+, *&"-*-(//%$%"+(&+(0"*&"-*&7'0*("*-%9 /%"'%* . %#, &"(' . '*+, 1'*, (), 7(), +%"(")*+, %($*("+$()1(")* &"-*1"%A2%#+%-*/1"#+(0"&7*(. 20$+&"#%*("*("3%$+%5$&+%* 7(/%*B@7OO-*%+*&7:<*CDDDF* a, & . &$(*%+*&7:<*CDEMF*G&7& . O"%&<* CDECF*GO")=7&"&=&$("<*CDDHI:*

K&=(")*("+0* &##01"+* +, %* /%%-(")* , &5(+'* 0/* $%2$%'%"+&9 +(3%'*0/*Q% . %$+%&* 4%*#&"*'1220'%*+, &+*'+1-(%-*, 6-$09 5(0"+* . &6*#0"+&("-()%'+(3%*%"86 . %'*#&2&57%*+0* , 6-$09 768%*"&+(3%*#077&)%":*

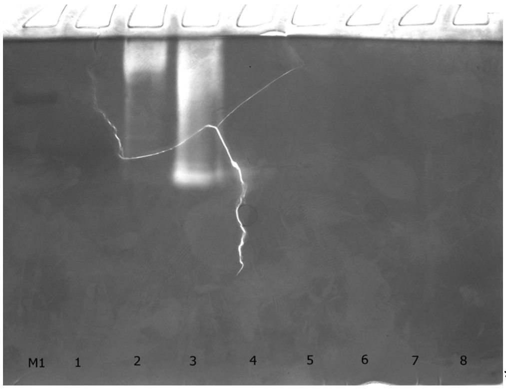

Figure 5. Proteolytic enzymes detection by gelatin zymography: M1 – plasmin (85 кДа); 1-8 – fraction number

!+*', 017-*5%*"0+%-*+, &+*#077&)%"076+(#*%"86 . %'*0/* . &9 $("%* , 6-$05(0"+'* , &3%*+, %* &5(7(+6*+0*#7%&3%*+, %* +$(27%9 , %7(A*0/*#077&)%"*1"-%$*#0"-(+(0"'*4, (#, *-0*"0+*-%"&9 +1$%*+, %*2$0+%("*:!"*+, ('*$%'2%#+<*+, %6*&$%*'(. (7&$*+0*#079 7&)%"&'%'*0/* , (), %$*&"(. &7'*4, (#, *27&6*&*$07%*("*+, %* 2, 6'(070)(#&7*$% . 0-%7(")*0/*#0"'%#+(3%*+(''1%':*K, %$%*('<* , 04%3%$<*&*-%/("(+%*-(//%$%"#%*("*+, %*#&+&76+(#* . %#, &"9 (' . *5%+4%%"*+, %*&503%*-()%'+(3%*#077&)%"076+(#*%$86 . %'* &"-*3%$+%5$&+%*#077&)%"&'%'*9*+, %*7&++%$*%"86 . %'*#&+&9 768%*+, %*#7%&3&)%*0/*+, %*'15'+$&+%*2$%-0 . ("&"+76*("*0"%* 2&$+*(#17&$*20("+*&"-*'15'+&"+(&776*("#&2&57%*+0* , 6-$09 768%* 7&$)%* '07157%* /$&) . %"+'* BP0++* &"-* d%$5<* CDDMI4, %$%&'*#077&)%"076+(#* 2$0+%&'%'*0/* , 6-$05(0"+'* #&"*#7%&3%*"&+(3%*#077&)%"*("* . &"6*20("+'*Bb1-%"'=&6&* %+*&7:<*CDDMI:*

`"*+, ('*5&'('<*+, %*"%A+*'+&)%*0/*01$*40$=*4&'*-%30+%-*+0* &"&768%*+, %*2$0+%("*/$&#+(0"'*05+&("%-*&/+%$*)%7*/(7+$&+(0"* #, $0 . &+0)$&2, 6*0/*+(''1%*%A+$&#+*0/* !"#$%&& '()*'+#0"*+, %* 2$%'%"#%*0/* #077&)%"076+(#* &#+(3(+6:* N##0$-(")*+0* 059

+&("%-*$%'17+'*'1 . . &$(8%-*("*5"?)'& .*+, ('*+62%*0/*2$09 +%076+(#*&#+(3(+6* 4&'*/01"-*+0*5%*2$%'%"+*("*'%3%"*%A9 & . ("%-*2%&=':* d%*$%3%&7%-* . &A(. 1 . *#077&)%"076+(#* &#+(3(+6*("*+, %*2%&=<*4, (#, *#0$$%'20"-*+0*+, ($-*/$&#+(0"* +, &+<*("*)%"%$&7<*('*%"+($%76*#0"'('+%"+*4(+, *+, %* -&+&*0/* 86 . 0)$&2, 6*&501+*+, %*2$%'%"#%*0/*+, %* . 0'+*%A2$%''%-* #7%&$*5&"-*&+*+, ($-*+$%=*0/*86 . 0)$& . :*

]&'%-*0"*7(+%$&+1$%*-&+&*BL&$=*%+*&7:<*CDDCF*b& . 1"-0*&"-* a$&6<*CDD^I*&"-*01$*04"*$%'17+'*4%*#&"*#0"#71-%*+, &+* #077&)%"076+(#* &#+(3(+6*("* E9f* 2%&='* . &6*("-(#&+%* +, %* 2$%'%"#%*("*+(''1%*%A+$&#+'*0/* !"#$%&& '()*'+##077&)%"&'%'<* +, %* . 07%#17&$*4%(), +'*0/*4, (#, *&$%*("*+, %*&3%$&)%*$&")%* 0/* fD*+0*YD*=U&<* 4, %$%&'*&#+(3(+6*("*M9Y*2%&='* . &6*5%* &''0#(&+%-* 4(+, * 704* . 07%#17&$* 4%(), +* #077&)%"076+(#* '%$("%*2$0+%&'%':*G0<* &* "1 . 5%$* 0/*&1+, 0$*"0+%*+, &+*("* #0"+$&'+*+0*, (), 9 . 07%#17&$*5&#+%$(&7*&"-*+(''1%*#077&)%9 "&'%'*0/* , (), %$*&"(. &7'*4, (#, ** . 07%#17&$*4%(), +'*&$%* /01"-*+0* 3&$6*/$0 . * fD*+0* ERD*=U&*#077&)%"076+(#*%"9 86 . %'*0/* . &$("%*("3%$+%5$&+%'<* &$%*#0 . 27%A*0/*('0%"9 86 . %'*4(+, * . 07%#17&$*4%(), +'*12*+0*fZ*=U&*Bg(. *%+*&7:<* CDDCF*G&7& . O"%&<*CDECI:*

Table 1. Proteolytic activity in protein fractions of *Parborlasia corrugatus*

5@A'&2B& A62('2)@(1C& "C(1D1(@&	;6"C(12E&EF 3 ?'6							
	.	,	=&	>&	/	G	8	H
SO77&)%"O9 76+(#*&#+(3(9 +6<** 1"(+'? .)* 2$0+%("**	C^:Ch D:RR*	CZ:Mh D:MR*	fC:Yh D:Zf*	EY:fh D:fR*	CD:E hD:R*	CRh D:RZ*	EY:Hh D:CH*	O*
K$62'("9 7(=%*&#+(3(9 +6<** µ . O7*. O "(+$O&"(9 7("%? . ("*x* .)*2$0+%("**	C:MhD :DM* *	O*	O*	O*	O*	O*	O*	O*

K, 1'<*01$*/("-(")'* . &6*("-($%#+76*("-(#&+%*+, %*2$%'%"#%* ("*+(''1%'*%A+$&#+*0/*("3%'+()&+%-* , 6-$05(0"+*%"86 . %'* 5%7O")(")*+0*+, %*'%$("%*#077&)%"076+(#*2$0+%&'%'*&'*4%77* &'*+0* . %+&770#077&)%"&'%'<*+&<*0/*#01$'%<*$%J1($%* . 0$%* -%+&(7%-*'+1-(%'*1(")*&22$02$(&+%*(", (5(+0$':*

N'*+$62'("'*&$%*2$("#(2&7*-()%'+(3%*2$0+%("&'%'*("* . 0'+* , 6-$05(0"+'*B@1#, ('%*%+*&7:<*CDEEI<*("*&--(+(0"*+0*#077&9)%"076+(#*&#+(3(+6* 4%*'+1-(%-*+$62'("97(=%*&#+(3(+6:*K, %* , 6-$076+(#* &#+(3(+6* 4&'* &''%'%-* 1'(")*+, %* '6"+, %+(#* '15'+$&+%'* c9]N2QN:* K&=(")* ("+0* &##01"+* +, &+* c9]N2QN*('*&7'0*'1(+&57%*'15'+$&+%*/0$*#6'+%("*2$0+%("&'%'* 4%*1'%-*T9ZM*9*&"(", (5(+0$*0/*+, ('*/& . (76*0/*%"86 . %'* +, &+* , &'*%"&57%-*1'*+0*%'+(. &+%*'%$("%*2$0+%&'%*&#+(3(+6<* 2&$+(#17&$76<*&#+(3(+6*0/*+$62'("97(=%*%"86 . %':*b%'17+'*0/*

[illegible]

[illegible]

[illegible]

CONCLUSION

[illegible]

Competing interests

[illegible]

References

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry* 72, 248-254.

Flood, J., Mayne, J., and Robinson, J.J. (2000). Identification and characterization of gelatin-cleavage activities in the apically located extracellular matrix of the sea urchin embryo. *Biochemistry and Cell Biology* 78, 455-462.

Fuchise, T., Sekizaki, H., Kishimura, H., Klomklao, S., Nalinanon, S., Benjakul, S., and Chun, B.-S. (2011). Simple Preparation of Pacific Cod Trypsin for Enzymatic Peptide Synthesis. *Journal of Amino Acids* 2011, 1-8.

Ghamari, M., Hosseininaveh, V., Darvishzadeh, A., and Talebi, K. (2014). Biochemical characterisation of the tissue degrading enzyme, collagenase, in the spined soldier bug, Podisus maculiventris (Hemiptera: Pentatomidae). *Journal of Plant Protection Research* 54.

, S.-K., Park, P.-J., Kim, J.-B., and Shahidi, F. (2002). *Journal of Biochemistry and molecular biology* 35, 165-171.

Moore, S., Stein, W.H (1954). A modified ninhydrin reagent for the determination of amino acids and related compounds. *Journal of Biological Chemistry* 211, 907–913.

Mott, J.D., and Werb, Z. (2004). Regulation of matrix biology by matrix metalloproteinases. *Current Opinion in Cell Biology* 16, 558-564.

Ostapchenko, L., Savchuk, O., and Burlova-Vasilieva, N. (2011). Enzyme electrophoresis method in analysis of active components of haemostasis system. *Advances in Bioscience and Biotechnology* 02, 20-26.

Park, P.-J., Lee, S.-H., Byun, H.-G., Kim, S.-H., and Kim, S.-K. (2002). *Journal of Biochemistry and molecular biology* 35, 576-582.

Peck, L.S., Webb, K.E., and Bailey, D.M. (2004). Extreme sensitivity of biological function to temperature in Antarctic marine species. *Functional Ecology* 18, 625-630.

Ramundo, J., and Gray, M. (2009). Collagenase for Enzymatic Debridement. *Journal of Wound, Ostomy and Continence Nursing* 36, S4-S11.

Rudenskaya, G.N., Kislitsin, Y.A., and Rebrikov, D.V. (2004). *BMC Structural Biology* 4, 2.

Salamonea, M., Cuttittab, A., Seiditac, G., Mazzolad, S., Bertuzzie, F., Ricordif, C., Ghersig, G (2012). Characterization of collagenolytic/proteolytic marine enzymes. *Chemical Engineering Transactions* 27, 1–6.

Songklanakarin, S.K. (2008). Digestive proteinases from marine organisms and their applications. *J Sci Technology* 30 (1), 37–46.

Wilkesman, J., and Kurz, L. (2009). Protease Analysis by Zymography: A Review on Techniques and Patents. *BIOT* 3, 175-184.

Xavier, L.P., Almeida Oliveira, M.G., Guedes, R.N.C., Santos, A.V., and De Simone, S.G. (2005). Trypsin-like activity of membrane-bound midgut proteases from Anticarsia gemmatalis (Lepidoptera: Noctuidae). *Eur J Entomol* 102, 147-153.

Cite this article as:

[illegible]