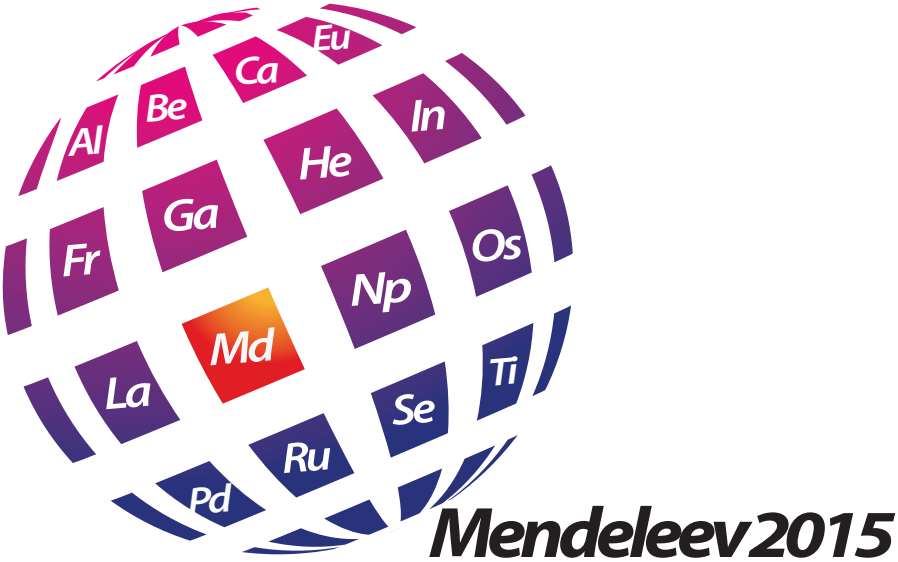
IX International conference of young scientists on chemistry „Mendeleev- 2015”



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**IX INTERNATIONAL CONFERENCE
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Section 4
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RECOMBINANT CATHEPSIN L FROM *TRIBOLIUM CASTANEUM*: ISOLATION AND PROPERTIES

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This research is devoted to recombinant cathepsin LNP_001164001 from *Triboliumcastaneum*, its isolation and properties. The goal is a structure-functional characteristic of the recombinant cathepsin L, cysteine peptidase from papain family C1.The red flour beetle (*Triboliumcastaneum*) is a species of beetle in the family *Tenebrionidae* and a worldwide pest of stored products, particularly food grains. Analysisof *T.castaneum* larvae gut transcriptome revealed the presence of twenty-five cysteine peptidases from C1 family.The highest level of expression in the gut of this insect pest was for the major cathepsin LNP_001164001, which is considered as the major digestive enzyme of this insect. [1] The diet of *T. castaneum* larvae consists mainly of cereals. The major storage proteins in cereals are prolamins that cause celiac disease in susceptible people. Given that, we can assume, that cathepsin L NP_001164001can be used as part of combination enzyme therapy of coeliac disease. Native cathepsin L isolated from the insect gut is extremely unstable. Therefore, the recombinant protein may facilitate investigation of this enzyme. In this research recombinant *T. castaneum* cathepsin L was expressed in *Pichia pastoris* as an inactive proenzyme, therefore the autocatalytic processing of procathepsin L was studied in detail *in vitro*.

Procathepsin L is stable at slightly alkaline pH, and the proregion protects the protein from the denaturing effect of neutral to alkaline pH. Proenzyme activation step consists of proteolytic cleavages of the proregion, autocatalytic processing of procathepsin L occurs under acidic conditions. [2] Based on that, the optimal conditions for processing are determined. Mature enzyme is characterized according to activity profiles in buffers of different pH, mass spectrometry analysis, electrophoretic mobility under native and denaturation conditions, inhibitor sensitivity and substrate specificity. Furthermore, comparative analysis with other cysteine cathepsins will be conducted.

[1]Martynov A. G., Elpidina E. N., Perkin L., Oppert B. Functional analysis of C1 family cysteine peptidases in the larval gut of Tenebriomolitor and Triboliumcastaneum. Martynov et al.BMC Genomics, 2015, vol 16, pp 75

[2]Ménard R., Carmona E., Takebe S., Dufour E., Plouffe C., Mason P., Mort J. S.Autocatalytic Processing of Recombinant Human Procathepsin L.The Journal of Biological Chemistry, 1998, vol 273(8), pp 4478-84

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