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CLONING AND EXPRESSION OF CHITINASE 19 FAMILIE FROM *D.CAPENSIS*

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Chitinases are divided into two main families. Family 18 chitinases are widely studied class of enzymes are presented in almost all groups of organisms and possess a broad spectrum of physiological functions. Fungal producers 18 family are used in agriculture for the protection of plants against plant pathogens and pests. Chitinases of family 19 (GH19) less studied, but their potential as a biocontrol agent can significantly exceed the chitinases family 18, since they are evolutionary proteins immune response of higher plants in response to infection by phytopathogens. Chitin-degrading activity determines practical use of chitinases as protective agents against pathogenic organisms such as fungi and insects. These properties allow the use of chitinases for biocontrol of plant pathogens and eliminate the step of treating with mutagenic pesticides of agricultural plants.

This article focuses on the cloning and expression of heterologous chitinase family 19 (Gh19) of the carnivorous plant *Drosera capensis*, that can effectively destroy the exoskeleton of an insect.

We were isolated RNA and DNA from plants obtained *IV vitro* by modified CTAB method [1]. The sequence of the chitinase gene was reconstruct by TAIL-PCR [2] and submission in the GenBank data base (MK093978). Gene length was 2,443 bp, including three introns. The predicted open reading frame composed of 978 nucleotides was confirmed by amplifying the coding sequence from the cDNA. Protein encoded by this sequence has a length of 325 amino acid residues and represents a classical chitinase having two domain and a signal peptide at the C-terminal region.

In this work was carried heterologous expression of full GH19 and GH19 without chitin-binding domain in different strains of *E.coli: Arctic Express (DE3*), *Rosetta™(DE3), Rosetta-gami™ 2(DE3)* и Shuffle *(DE3*). However, soluble form of protein wasn't obtained in any of the strains. To restore the native structure GH19 from inclusion bodies, refolding protocol was optimized. Refolding of inclusion bodies was carried out in the system of oxidized and reduced glutathione

To the resulting homogeneous chitinase was determined optimum temperature and pH, which was 55 °C and 4,5 respectively. Under optimal conditions, the activity of chitinase for colloidal chitin was 0,56 U/mg for a full-size chitinase and 0,14 U/mg for a form without a chitin-binding domain. Nonspecific activity for other polysaccharides (microcrystalline cellulose, xylan, carboxymethylcellulose) is not detected.

In this way, the work was carried out a directed search and cloning of chitinase 19 family from *Drosera capensis*. Primary biochemical characteristics are described, and the optimum pH and temperature are determined. The set of characteristics of the obtained enzyme demonstrates the relevance of further study of fungicidal and phytoprotective properties, in order to create biological products for the control of phytopathogens.

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REFERENCES

1) Fleischmann A., Heubl G. Overcoming DNA extraction problems from carnivorous plants // An. del Jardín Botánico Madrid. 2009. Vol. 66. № 2. P. 209–215.
2) Jia X., Lin X., Chen J. Linear and exponential TAIL-PCR: a method for efficient and quick amplification of flanking sequences adjacent to Tn5 transposon insertion sites // AMB Express. 2017. Vol. 7. №. 1. P. 195–203.
3) Moghadam M., Ganji A., Varasteh A., Falak R., Sankian M. Refolding process of cysteine-rich proteins: Chitinase as a model Rep // Biochem. Mol. Biol. 2015. Vol. 4, № 1, P. 19–24.