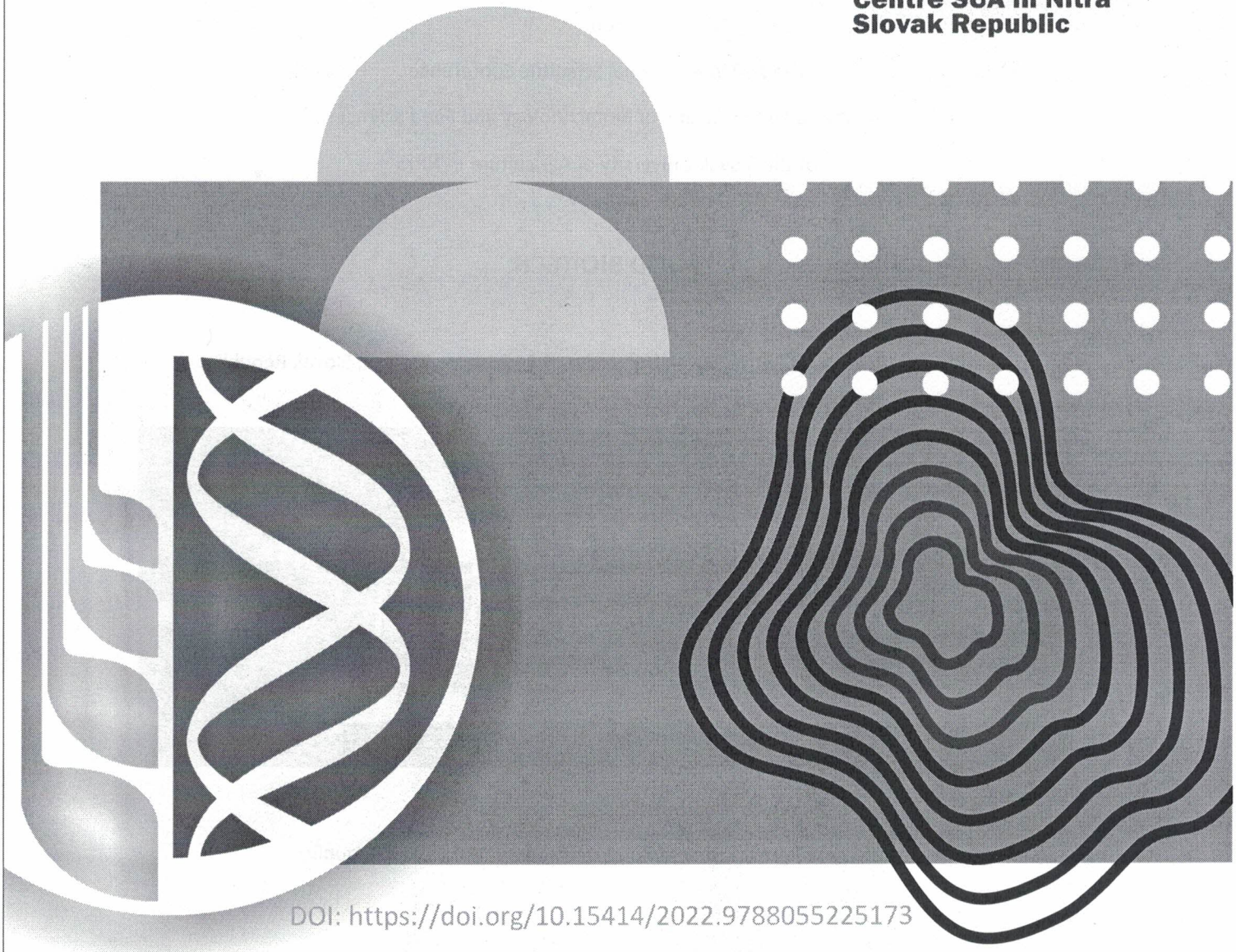
FOOD | BIO | TECH CONFERENCE 2022

15th International scientific conference

OCTOBER 5-6

AgroBioTech Research Centre SUA in Nitra Slovak Republic



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BOOK OF ABSTRACTS

FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCES

SLOVAK UNIVERSITY OF AGRICULTURE IN NITRA

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Slovak University of Agriculture in Nitra
Nitra, SLOVAK REPUBLIC

BOOK OF REVIEWED ABSTRACTS

from

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of the Slovak University of Agriculture in Nitra

FOOD|BIO|TECH

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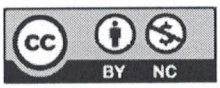

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THIOREDOXIN FUSION TAG IMPROVES PROTEIN YIELD OF RECOMBINANT *DROSERA BINATA* GLUCANASE

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The production of soluble recombinant proteins in *Escherichia coli* remains one of the major bottlenecks of prokaryotic expression systems. The addition of fusion tags to enhance the solubility of the produced protein is one of the best solutions for expressing enzymatically active eukaryotic proteins. In our work, we over-expressed the basic β-1,3-glucanase from the carnivorous plant *Drosera binata* in *Escherichia coli* cells. Two forms of enzymatically active protein were produced, which differ in the presence (DbGluc+Trx, ~50 kDa) or absence (DbGluc-Trx, ~30 kDa) of the thioredoxin fusion tag. Both forms of β-1,3-glucanase also contain the 6xHis-tag sequence for purification by affinity chromatography. Successful production of both forms of recombinant β-1,3-glucanase was detected by SDS-PAGE and the presence of both forms of β-1,3-glucanase was confirmed by on-gel detection of Ni-NTA conjugated fluorescence dye signal. Although both forms of β-1,3-glucanase showed similar specific activity (68.12 $U.mg^{-1}$ DbGluc+Trx and 64.61 $U.mg^{-1}$ DbGluc-Trx), thioredoxin fusion tag increased protein yield of β-1,3-glucanase two-times during purification on Ni-NTA agarose. We estimated that 69% of total enzymatic activity was preserved after purification of DbGluc+Trx, in comparison with DbGluc-Trx, where β-1,3-glucanase retained only 31% of its activity. Thioredoxin fusion tag significantly increased the yield of recombinant β-1,3-glucanase and its presence during purification steps is crucial for sufficient transgene production.

Keywords: *Drosera binata* β-1,3-glucanase, Ni-NTA purification, thioredoxin fusion tag

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