P6.17 - EVALUATION OF OCHRATOXIN A (OTA) INTERACTION WITH RECOMBINANT DOMAIN II OF BOVINE SERUM ALBUMIN TOWARD THE DEVELOPMENT OF NEW OTA EXTRACTION PLATFORMS

Tatiana Q. Aguiar [1,2(*)], ***Tânia Leal*** [3], ***Diana G. Rodrigues*** [1], ***Luís Abrunhosa*** [1,2], ***Carla Oliveira*** [3], ***Lucília Domingues*** [1,2]

[1] *CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[3] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[(*)] *e-mail: tatiana.aguiar@deb.uminho.pt*

Keywords: Ochratoxin A; Bovine serum albumin; Domain II; Interaction; Mycotoxin capture.

ABSTRACT

Ochratoxin A (OTA) is a mycotoxin that raises food and feed safety concerns, existing legal limits worldwide for its presence in foodstuffs and beverages. Immuno-affinity columns (IACs) are typically used to capture, clean-up and pre-concentrate OTA from food samples before quantification by high- performance liquid chromatography, but their price limits their application. Serum albumins form stable complexes with OTA, emerging as cheaper alternatives to the antibodies used in IACs. They are composed of three globular domains, being the principal OTA binding site located within the domain II. Containing only 6 of the 17 disulfide bonds present in albumins, this domain should be more efficiently produce by bacteria than entire albumins.

This work envisioned the recombinant production of the bovine serum albumin (BSA) domain II in Escherichia coli, the study of is interaction with OTA and its evaluation as ligand receptor for developing new OTA extraction platforms. For that, this domain was cloned in fusion with His6 tag (BDII) or with thioredoxin A (Trx)-His6 tag-TEV cleavage site (TrxBDII), and produced using BL21 and Origami 2 DE3 strains. The improved cytoplasmic oxidizing environment of Origami 2 allowed the best production yield (18-24 mg purified protein/L culture) and fusion with Trx slighlty improved the stability of BDII. Fluorescence quenching studies indicated weaker interaction of OTA with TrxBDII than with the entire BSA, but no significant differences between TrxBDII and BDII. Circular dichroism spectroscopy confirmed that OTA induced conformational changes in TrxBDII, leading to a slight loss in the α-helical content. Immobilized TrxBDII was finally used to capture OTA from buffered solutions, allowing full retention of the mycotoxin followed by recovery upon elution.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and project MycoProAffinity 2022.03438.PTDC (https://10.54499/2022.03438.PTDC).