

# **Nanobody-Alkaline phosphatase Fusion Protein-Based Enzyme-Linked Immunosorbent Assay for One-Step Detection of Ochratoxin A in Rice**

**Zhichang Sun <sup>†</sup>, Xuerou Wang <sup>†</sup>, Qi Chen, Yonghuan Yun, Zongwen Tang and Xing Liu <sup>\*</sup>**

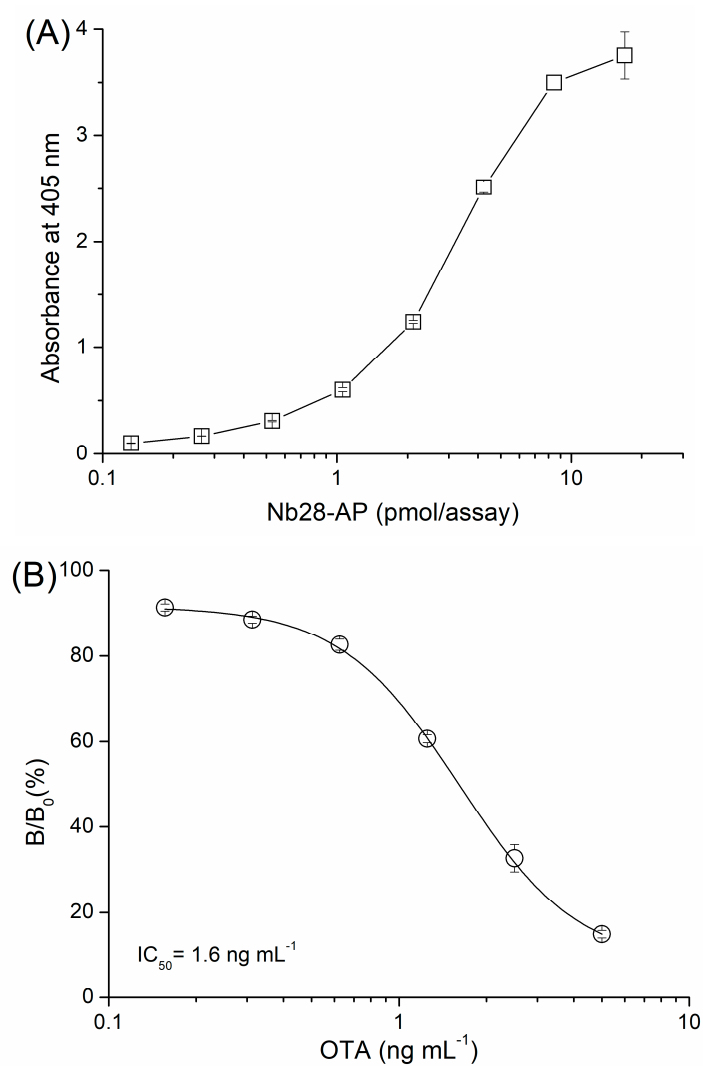
College of Food Science and Technology, Hainan University, 58 Renmin Avenue, Haikou 570228, China; 13919003678@163.com (Z.S.); w490487@126.com (X.W.); qichen@hainu.edu.cn (Q.C.); yunyonghuan@foxmail.com (Y.Y.); tzwcx@163.com (A.T.)

<sup>\*</sup> Corresponding author: xliu@hainu.edu.cn; Tel.: +86-898-6619-3581

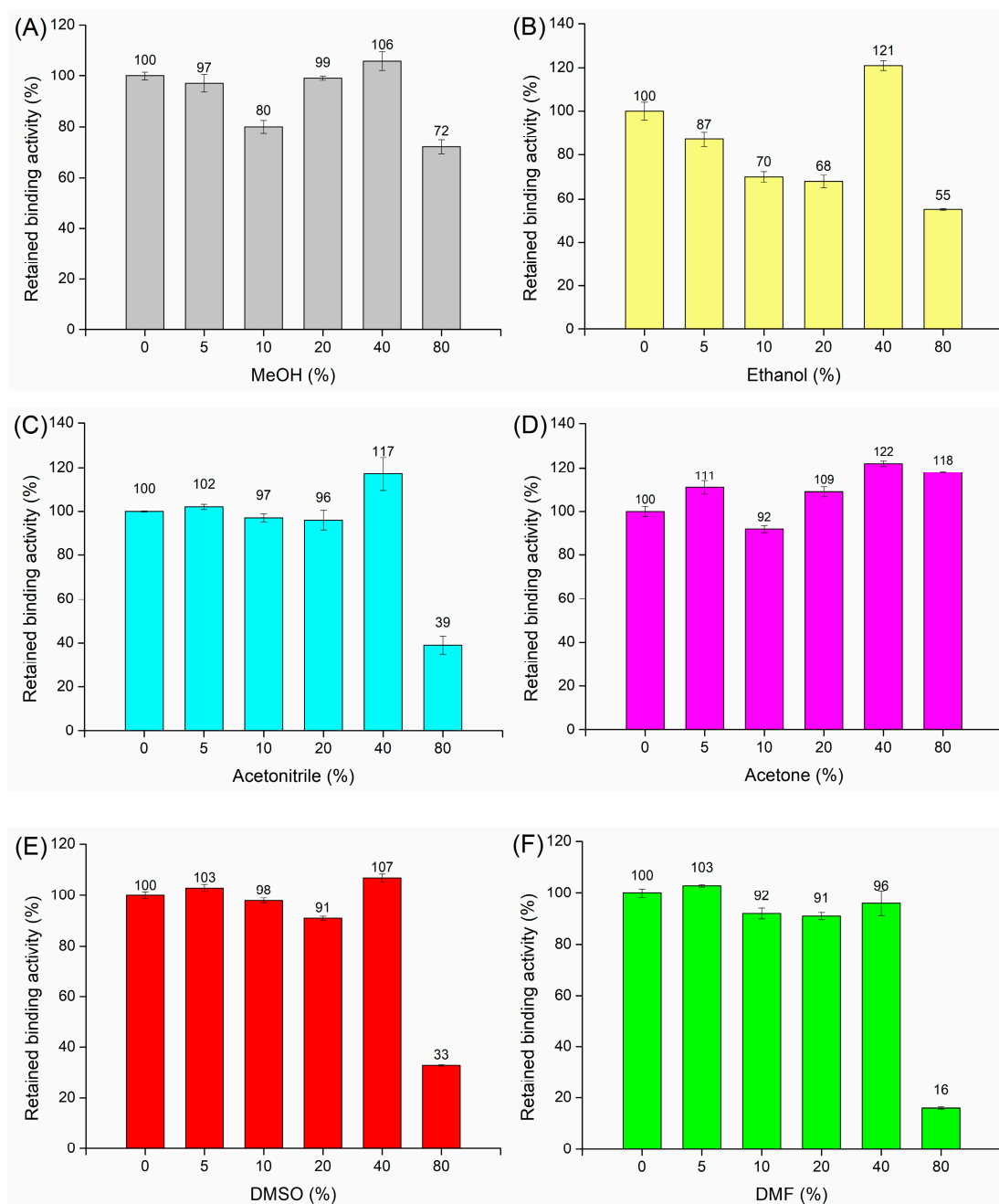
<sup>†</sup> These authors contributed equally to this work.

## **Table of contents**

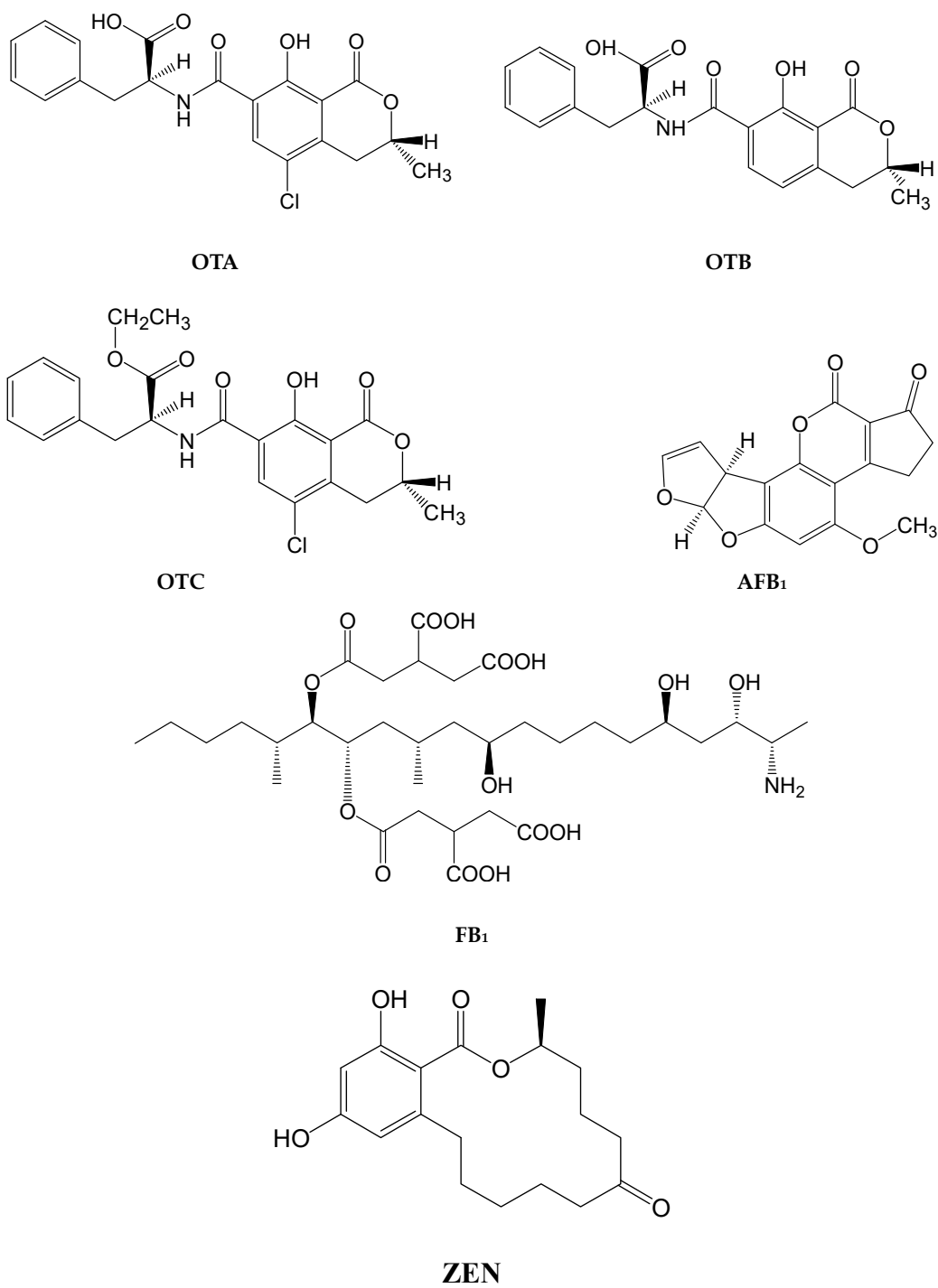
<b>Figure S1</b> .....	S-1
<b>Figure S2</b> .....	S-2
<b>Figure S3</b> .....	S-3
<b>Figure S4</b> .....	S-4



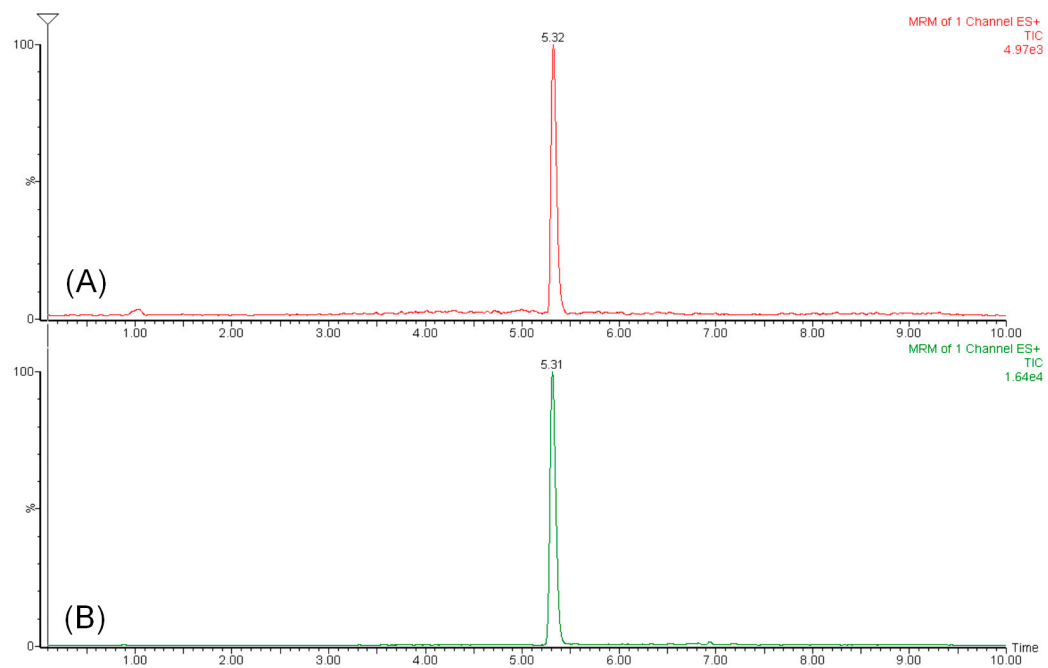
**Figure S1.** AP enzymatic activity and anti-OTA reactivity analysis of Nb28-AP. **(A)** Dose-response curve for AP enzymatic activity of Nb28-AP by colorimetric analysis. **(B)** Indirect competitive inhibition curve using Nb28-AP by colorimetric analysis. The error bars represent the standard deviation of three independent experiments.



**Figure S2.** The solvent tolerance of Nb28-AP for methanol (A), ethanol (B), acetonitrile (C), acetone (D), DMSO (E), and DMF (F). PBS buffers containing each organic solvent at different concentrations (0%, 5%, 10%, 20%, 40%, and 80%) were used to dilute Nb28-AP, and 100  $\mu$ L of the diluent was added into the wells coated with OTA-BSA. The bound Nb28-AP was detected by adding 100  $\mu$ L of pNPP substrate solution. The error bars represent the standard deviation of three independent experiments.



**Figure S3.** The chemical structures of OTA, OTB, OTC, AFB<sub>1</sub>, FB<sub>1</sub>, and ZEN.



**Figure S4.** LC-MS/MS analysis of the OTA-contaminated rice sample (A) and the OTA standard (B).