# Supplementary Materials: Truncated Expression of a Carboxypeptidase A from Bovine Improves Its Enzymatic Properties and Detoxification Efficiency of Ochratoxin A

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## 1. M-CPA effectively degraded OTA in vitro

Methods: The in vitro gastrointestinal model simulating the digestive processes of the stomach and small intestine was used as the methods described by Jiang et al (2010). Simulated gastric juice: 0.8 g of pepsin and 4.375 g of NaCl, dissolved in 250 mL of distilled water, adjusted to pH 1.2 with hydrochloric acid. Simulated intestinal juice: 0.143 g trypsin and 0.857 g bile, dissolved in 100 mL of 0.1 mol/L $NaHCO_3$ solution with pH 6.0.

For the gastric stage, 1.8 mL of gastric juice was added to the tube sets containing OTA (2 μg/mL) and M-CPA (10 U/mL)/S-CPA (10 U/mL), and the mixtures were rotated for 3 h and then collected 0.5 mL mixture every 1 h for further survival OTA extraction.

In the intestinal digestion stage, 1.8 mL of intestinal juice was added to the remaining tubes and incubated with rotation for another 3 h, which was then taken out for further OTA and OT$\alpha$ analysis every one hour.

### *1.1 The reference of method:*

Jiang, L., Olesen, I., Andersen, T., Fang, W. & Jespersen, L. (2010) Survival of Listeria monocytogenes in Simulated Gastrointestinal System and Transcriptional Profiling of Stress- and Adhesion-Related Genes, Foodborne Pathogens And Disease. 7, 267-274.

### *1.2. Results*

An *in vitro* simulated gastrointestinal digestion model was used to evaluate whether M-CPA can stably degrade OTA in the gastrointestinal digestive environment. M-CPA and S-CPA were incubated with OTA in a simulated gastrointestinal digestion model for 3 h, and TLC and HPLC analysis was used to detect the degradation of OTA during different simulated digestion processes. The results of Fig.4 and Table 1 showed that OTA was slightly degraded *in vitro* digestive processes (degradation rate of 19.70%), and the degradation rates of OTA by S-CPA and M-CPA were 20.80% and 71.29% respectively. These results indicated that S-CPA was unstable in the simulated gastrointestinal environment, while M-CPA could tolerate gastrointestinal digestion and maintain a good OTA degradation ability.

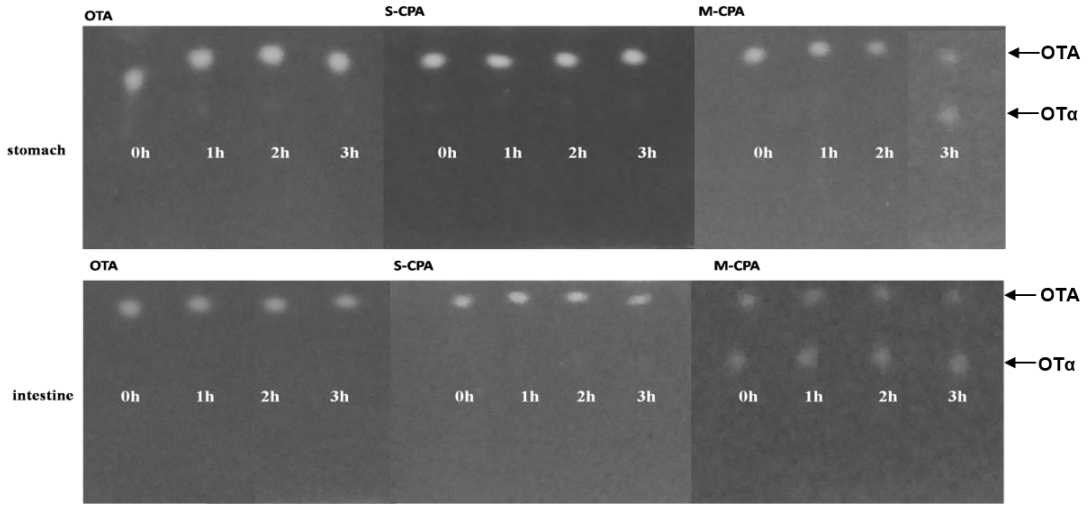


**Figure S1.** Thin layer chromatography of the recombinant M-CPA tested for OTA degradation during digestion in simulating gastrointestinal tract.

**Table S1.** Detoxification of OTA by M-CPA and S-CPA enzymes against control, during simulating gastrointestinal process.

| Procedure | Digestion time (h) | CK | S-CPA | M-CPA |
|---|---|---|---|---|
| Stomach | 0 | 0% | 0% | 0% |
| | 1 | 9.98% | 14.97% | 21.08% |
| | 2 | 8.24% | 15.89% | 44.37% |
| | 3 | 15.08% | 16.47% | 57.51% |
| Small intestine | 1 | 16.06% | 17.44% | 64.73% |
| | 2 | 18.25% | 18.41% | 65.77% |
| | 3 | 19.70% | 20.80% | 71.29% |

## 2. Gene sequence and amino acid sequence of the CPA

### *2.1. GeneBank ID: NM_174750.2*

>NM_174750.2 Bos taurus carboxypeptidase A1 (CPA1), mRNA
AGCTGACCTTCCCAACTGACTGCAGCATGCAGGGGCTGCTGATTTTGAGTGTGCTGCTGGG
GGCTGCCCTTGGCAAAGAGGACTTTGTGGGCCACCAGGTGCTCCGAATCACTGCCGCCGAT
GAGGCCGAGGTGCAGACGGTGAAGGAGCTGGAGGACCTGGAGCACCTGCAGTTGGACTTC
TGGAGGGGCCCTGGCCAGCCAGGCTCCCCCATCGACGTCCGAGTGCCCTTCCCCAGCCTCC
AGGCTGTTAAAGTCTTCCTGGAAGCCCATGGCATCAGATACAGGATCATGATCGAGGACGT
GCAGTCCCTGCTAGACGAGGAGCAGGAGCAGATGTTCGCCTCCCAGAGCCGGGCCCGCAG
CACCAACACATTTAACTACGCCACCTACCACACCCTGGATGAGATCTATGACTTCATGGAC
CTGCTGGTGGCCGAGCACCCACAGCTTGTCAGCAAACTCCAGATTGGCAGAAGCTATGAAG
GCCGTCCCATCTACGTGCTGAAGTTCAGCACTGGGGGAAGCAACCGTCCAGCCATCTGGAT
CGACTTAGGCATCCATTCCAGGGAGTGGATCACCCAGGCCACTGGGGTCTGGTTTGCAAAG
AAGTTCACAGAAGACTATGGCCAGGACCCGAGTTTCACCGCCATTCTTGACAGCATGGACA
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TGGGAACTTCAAGGCCTTCCTCTCCATCCACAGCTACTCCCAGCTCCTCCTCTATCCCTATG
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TGAGGCCCTGAAGTCTCTGTATGGGACCAGCTACAAGTATGGCAGCATCATCACAACAATT
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CCTTTGAACTCCGGGACACGGGGCGCTATGGCTTCCTGCTGCCAGCCTCCCAGATCATCCCC
ACAGCCCAGGAGACGTGGCTGGGGGTTCTGACCATCATGGAGCACACGTTGAATAACCTCT
ACTGACCCAGCCCTCCAGCACTCTTTTCCTCCTCCTCTTCAGCCCTACCCAGGCAACCAAAT
AAAGTCTGAGAGTACATGG

*2.2. GeneBank ID: CAA83955.1*

>CAA83955.1 carboxypeptidase A [Bos taurus]
MQGLLILSVLLGAALGKEDFVGHQVLRITAADEAEVQTVKELEDLEHLQLDFWRGPGQPGSPID
VRVPFPSLQAVKVFLEAHGIRYRIMIEDVQSLLDEEQEQMFASQSRARSTNTFNYATYHTLDEIYD
FMDLLVAEHPQLVSKLQIGRSYEGRPIYVLKFSTGGSNRPAIWIDLGIHSREWITQATGVWFAKKF
TEDYGQDPSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGF
GKAGASSSPCSETYHGKYANSEVEVKSIVDFVKDHGNFKAFLSIHSYSQLLLYPYGYTTQSIPDKTE
LNQVAKSAVAALKSLYGTSYKYGSIITTIYQASGGSIDWSYNQGIKYSFTFELRDTGRYGFLLPASQI
IPTAQETWLGVLTIMEHTVNNLY