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Supplementary Material

Expression and Characterization of Rice-produced Recombinant Porcine Lactoferrin and its Antioxidant Activities

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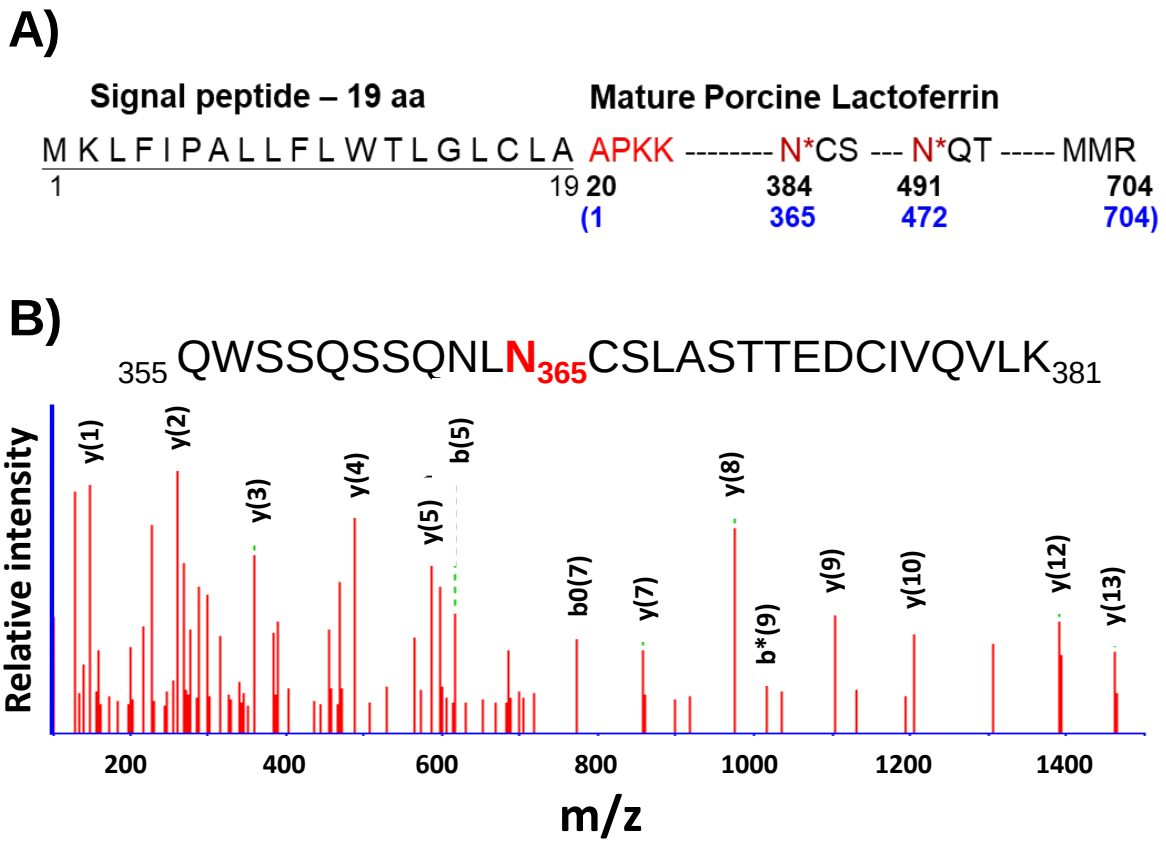
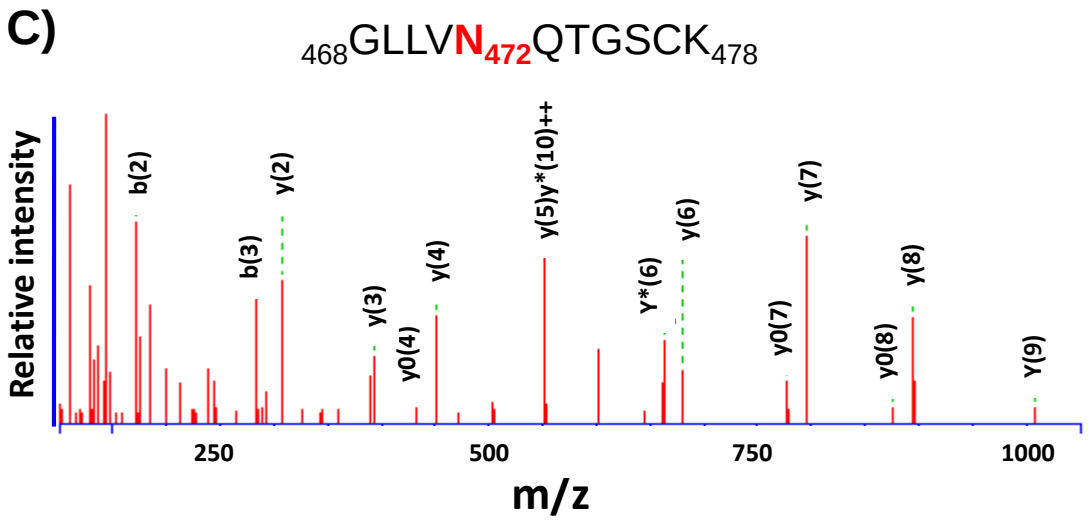
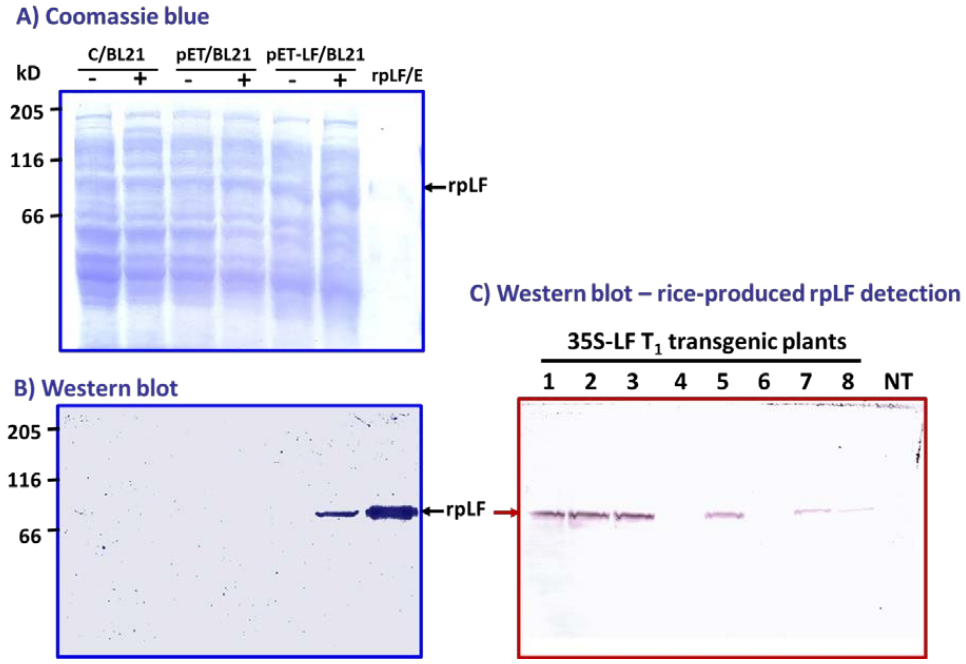
Article History	Received: March 15, 2020	Revised: May 12, 2020	Accepted: May 22, 2020



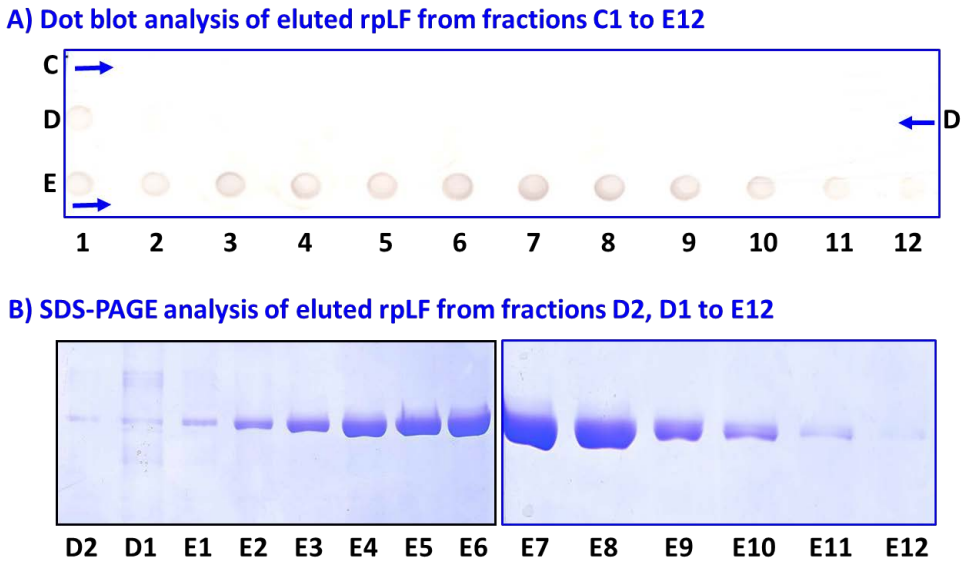
Supplementary Fig. 1 cont.....



Supplementary Fig. (1). Results of the Edman degradation assay and the identification of N-glycosylation sites of purified rpLF by LC-MS/MS assay. The N-glycosylation site was analyzed using trypsin digestion followed by the addition of PNGase F (N-glycosidase F). Both deglycosylated and non-deglycosylated samples were subjected to LC-MS/MS analysis. A) Mature rpLF starts with APKK. The 19 signal peptide sequence of rpLF and its two predicted N-glycosylation sites at N365 and N472 are indicated. B) The peptide 355-381 "QWSSQSSQNLNCSLASTTEDCIVQVLK" with m/z 1028.8156(3+) was identified as a deamidated peptide (+1 Da at N11), indicating its original glycosylation. The MS/MS spectrum showing the identified b- and y-ion peaks with their relative intensity. C) The peptide 468-478 "GLLVNQTGSCK" with m/z 589.2974(2+) was identified as a deamidated peptide, indicating its original glycosylation. The MS/MS spectrum showing the identified b- and y-ion peaks with their relative intensity.



Supplementary Fig. (2). Analysis of antibody specificity against rpLF. A) SDS-PAGE of proteins extracted from cultures of E. coli BL21 (C/BL21), cultures with pET30a plasmid in BL21 (pET/BL21) and cultures with pET30a-LF plasmid in BL21 (pET-LF/BL21) either induced (+) or not-induced (-) with IPTG. The rpLF (1 µg) purified from pET-LF/BL21 E. coli culture (rpLF/E) was loaded as a positive control. B) Detection of E. coli-expressed rpLF by anti-LF antiserum raised in a rabbit. Results show anti-LF antiserum recognize rpLFs with high specificity. C) Example of the detection of rice-expressed rpLF from eight 35S-LF T1 transgenic plants. Two plants (lanes 4 and 7) showing no signals were segregated wild-type plants. Results show this antibody recognize rice-expressed rpLFs with high specificity as well.



Supplementary Fig. (3). Fractionation and detection of rpLF through a Heparin-Sepharose column with an AKTA purifier 10 system. A) Dot-blot analysis of eluted proteins from fractions C1 to E12 using anti-LF antibody for detection of rpLF. Results show the rpLF starts elution from E1 and peak at E6 to E8. Arrows indicate the directions of sample collection. B) SDS-PAGE analysis of eluted rpLF from fractions D2 to E12 and stained with Coomassie blue. The fractions from E4 to E9 were collected for further analysis.

Supplementary Table 1. The analysis gradient used in the LC-MS/MS assay.

Time (min)	Mobile phase A (%) -0.1% FA	Mobile phase B (%) -0.1% FA in 95% ACN
0	95	5
2	95	5
45	60	40
50	10	90
60	10	90
65	95	5
70	95	5