Supplementary information for

Antifungal Activity, Structural Stability, and   
Immunomodulatory Effects on Human Immune Cells of   
Defensin from the Lentil *Lens culinaris*

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**Table S1.** List of overlapping primers.

|  |  |
| --- | --- |
| **№** | **primer sequences (5’-3’)** |
| s1 | CCTCGACGCTAACCTGGCCGGATCTATGAAAACTTGTGAACACTTGGCTGATACCTA |
| s2 | CTTGGCTGATACCTACCGTGGAGTTTGTTTCACTAATGCTAGTTGTGACGATCACTGTAAAAA |
| ans3 | CATTTCCAATTATGACACGTGCCTGATATTAGATGTGCTTTATTTTTACAGTGATCG |
| ans4 | GGTGCTCGAGAGAATTCGCGGATCCTTAACAGTTTTGGGTACAGAAGCATTTCCAATTATGACAC |

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**Figure S1.** MALDI mass spectra of the recombinant defensins.

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**Figure S2.** (**A**) Comparison of the amino acid sequences of two legumes defensins – lentil Lc-def and pea Psd1. Identical and similar amino acids are shown in green and blue, respectively. %I, percentage of sequence identity. (**B**) ELISA with lentil Lc-def and pea Psd1 using rabbit polyclonal anti-Lc-def antibodies. Error bars represent standard deviation between technical replications. (**C**) SDS-PAGE analysis or (**D**) immunoblotting with rabbit polyclonal anti-Lc-def antibodies of Lc-def in the absence (**1**) or present (**2**) of BME and lysate of *E. coli* cells (**3**) expressing the fusion protein 8His-Trxl-Lc-def (MW 19kDa).

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**Figure S3.** (**A**) The growth of the clinical isolate of *C. albicans* in the presence of different concentrations of lentil lipid transfer protein Lc-LTP2 (100, 50, 25, 12.5, 6.25, 3.125 and 1.56 μM). Asterisk (red) shows optical density of the control without peptide. (**B**) Hemolytic assay of different concentrations of Lc-def and melittin as positive control. Error bars represent standard deviation between technical replications.

Diagram

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**Figure S4.** (**A**) Effect of heating on the sensitivity of Lc-def to digestive enzymes as detected by means of RP-HPLC on Luna C18 column (5 μm, 250 × 4.6 mm; Phenomenex) at a flow rate of 0.5 mL/min, using a linear gradient of acetonitrile concentration from 5 to 80% for 60 min in 0.1% trifluoroacetic acid (0 ‘ and 120’ p— pepsin digestion during 10 s and 120 min; 120’ and 24 h p+ch —subsequent digestion by mixture trypsin/α-chymotrypsin during 120 min and 24 h). (**B**) MALDI mass spectra of the RP-HPLC fractions (from 28, 31 and 33 min) of Lc-def digests.