cDNA Cloning, Heterogeneous Expression and Biochemical Characterization of a Novel Trypsin-Like Protease from *Nilaparvata lugens*

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Z. Naturforsch. **65 c**, 109–118 (2010); received August 17/September 24, 2009

A reverse transcription-polymerase chain reaction (RT-PCR) strategy was used to clone diverse trypsin-like protease gene transcripts from midguts of the brown planthopper *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Six individual trypsin-like protease transcripts were identified. On the basis of one nucleotide sequence of the six clones, a full-length cDNA sequence (1902 bp) was obtained by rapid amplification of cDNA ends (RACE). The cDNA contained an 1128-bp open reading frame encoding a putative protein of 375 amino acids with typical features of the trypsin-like protease. Heterogeneous expression of the coding sequence for the mature peptide in *Escherichia coli* cells showed that the expressed protease with a molecular weight of 27.0 is active, for its BApNAse activity assayed by using BApNA (*N*-benzoyl-D,L-arginine-*p*-nitroanilide) as substrate. The protease had its maximum activity at pH 8.0 and 35 °C. A much better stability was observed at pH values above 4.0 and temperatures below 40 °C. The enzyme was strongly inhibited by serine protease inhibitor. The trypsin-like protease is therefore likely one of the major digestive proteases responsible for protein hydrolysis in *N. lugens* gut, and multiple gene families encoding digestive proteases may help in adaptation of this sap-sucker to different rice varieties.

Key words: Trypsin-Like Protease, Heterogeneous Expression, BApNA (*N*-Benzoyl-D,L-arginine-*p*-nitroanilide)