SHORT COMMUNICATION

PCR Detection of the 14.5 Antibacterial NlpC/P60-Like *Dermatophagoides pteronyssinus* Protein in *Dermatophagoides farinae* (Acari: Pyroglyphidae)

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ABSTRACT House dust mites produce antibacterial proteins suppressing bacterial growth. The 14.5-kDa bacteriolytic protein (UniProtKB Q8MWR6) has been known in *Dermatophagoides pteronyssinus* Trouessart. We have applied polymerase chain reaction and reverse transcription-PCR to detect a homologous gene sequence coding for a Q8MWR6-related protein in *Dermatophagoides farinae* (Hughes) using genomic DNA and total RNA, respectively. The resulting PCR product of expected size, 243 bp, was obtained from both *Dermatophagoides* spp., while no amplification was achieved from stored product mite samples. Sequence of the gene fragment from *D. farinae* showed 83% similarity to the previously described one in *D. pteronyssinus*. Successful amplification of the expected product from cDNA generated with oligo-dT primer implies that the NlpC/P60-like protein in *Dermatophagoides* mites is of eukaryotic or mite origin.

KEY WORDS antibacterial proteins, allergen, house dust mite, digestion, NlpC/P60

House dust mites are suggested to use nutrients originating from debris of skin, nails, and hair. However, the house dust mites may feed on microorganisms growing on the organic debris and use nutrients from the microbial cells or interact with them in the organic debris degradation (Colloff 2009). Valerio et al. (2005) described bacterial community in the house dust mites based on 16S rRNA gene, while endotoxins produced by gram-negative bacteria were found in the vaccines prepared from house dust mites (Trivedi et al. 2003, Yella et al. 2011). Recently, the bacterial adjuvant compounds from the house dust mites as well as intrinsic biological properties of house dust mite allergens are suggested as determining factors for the stimulation of innate immune systems (Jacquet 2011).

Mathaba et al. (2002) suggested that *Dermatophagoides pteronyssinus* (Trouessart 1897) possess several bacteriolytic enzymes. The bacteriolytic enzymes can correspond to the digestive lysozymes present in astigmatid mites including *D. pteronyssinus* and *Dermatophagoides farinae* Hughes 1961 (Childs and Bowman 1981, Erban and Hubert 2008). Mathaba et al. (2002) purified and analyzed a 13.8-kDa protein (14.5 kDa according to deduced amino acid sequence; UniProtKB Q8MWR6) from the faeces fraction (SGME) of *D. pteronyssinus*.

Although the protein showed bacteriolytic activity and molecular mass similar to lysozymes, the sequence analysis indicated similarity to prokaryotic P60 proteins (Mathaba et al. 2002). In the current study, we identified a homologous protein in *D. farinae*.

Materials and Methods

D. farinae and *D. pteronyssinus* were reared in the laboratory cultures facility at Crop Research Institute, Prague, Czechia, as described in Erban and Hubert (2008). In addition, we tested the presence of the protein-coding sequence in stored product mites: *Acarus siro* L. 1758, *Aleuroglyphus ovatus* Troupeau

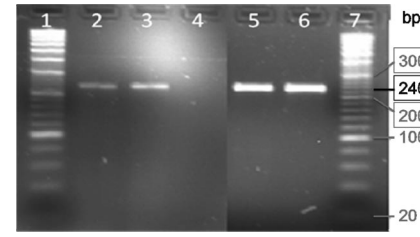

Fig. 1. PCR products in 2.5% agarose gel obtained with D1 F/D1 R primers from different templates. Lines: 1 and 7—PCR Low Ladder Set (Cat. No. D7808, Sigma Aldrich); 2—cDNA from *D. farinae* prepared with random hexamers for the first strand synthesis; 3—cDNA from *D. farinae* with poly-dT for the first strand synthesis; 4—direct amplification of *D. farinae* RNA with D1 F/D1 R primers (control); 5—*D. farinae* DNA; 6—*D. pteronyssinus* DNA (positive control).

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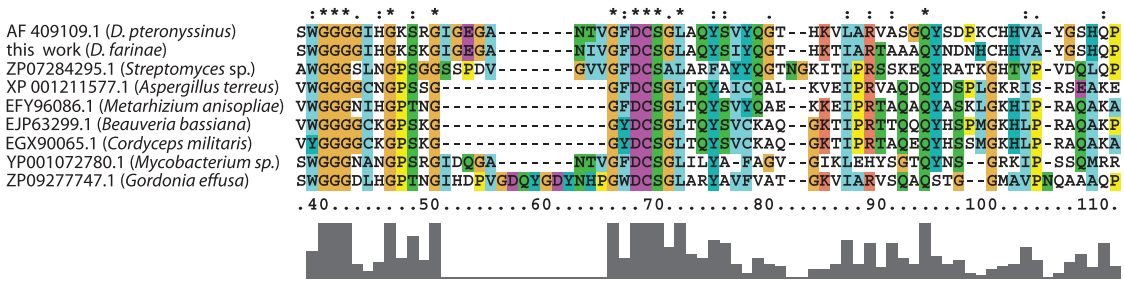

Fig. 2. Alignment of a partial sequence of 14.5-kDa bacteriolytic enzyme in *D. pteronyssinus* (Mathaba et al. 2002), with deduced amino-acid sequences of the gene fragment retrieved from *D. farinae* (this work), and corresponding parts of the closest homologs found in bacteria (*Streptomyces* sp., *Mycobacterium* sp., *Gordonia effusa*) and fungi (*Aspergillus terreus*, *Metarhizium anisopliae*, *Beauveria bassiana*, and *Cordyceps militaris*). Numbering according to the *D. pteronyssinus* protein. (Online figure in color.)

1878, *Sancassania berlesei* Michael 1903, *Tyrophagus putrescentiae* Schrank 1781, *Tyroborus lini* Oudemans 1924, *Blomia tropicalis* Van Bronswijk, De Cock and Oshima, 1974, *Carpoglyphus lactis* L. 1758, *Chortoglyphus arcuatus* Troupeau 1879, *Glycyphagus domesticus* De Geer 1778, *Lepidoglyphus destructor* Schrank 1871, *Aëroglyphus robustus* Banks 1906, see Kopecky et al. (2013) for rearing conditions and sampling. The mites were surface sterilized by bleach and ethanol solution 1:1 and further washed three times with ddH_2O. Homogenization was performed using Potter–Elvehjem glass homogenizer (Kartell Labware division, Noviglio, Italy); briefly, a 100-mg sample of the whole mite body was homogenized. The DNA was purified from the homogenates using FastDNA Spin kit for Soil (MP Biomedicals, Illkirch, France). The DNA was stored in freezer at −40°C until use. Total RNA was purified after homogenization in TRI Reagent (Sigma–Aldrich, St. Louis, MO) according to the manufacturer's instructions. Fragment of Q8MWR6 protein-coding sequence was PCR amplified using degenerate primers D1 F (5′-TCACAAATTGGT GTTCCWTA-3′, position 91–110 according to *D. pteronyssinus* sequence, GenBank AF409109) and D1 R (5′-ACCGAAAAATACTAAATCWCC-3′, position 304–324 according to AF409109). cDNA was obtained using Illustra Ready-To-Go reverse transcript-polymerase chain reaction (RT-PCR) Beads (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) using either oligo-dT primer or random hexamers.

The PCR reaction mixture contained in a total volume of 50 μl: 1× reaction buffer, 0.4 μM of each primer, 200 μM of dNTP, 50 ng of template DNA or cDNA, and 2.5 U of *Taq*-Purple DNA polymerase (Top-Bio, Prague, Czechia). The PCR program of C-1000 Thermal Cycler (BIORAD, Hercules, CA) included a denaturation step of 3 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 54°C, 30 s at 72°C, and a final elongation step of 5 min at 72°C. The PCR products of expected size were purified using QIAquick PCR purification kit (QIAGEN, Valencia, CA), cloned into pDrive Cloning Vector (QIAGEN PCR Cloning^{plus} Kit) according to the manufacturer's instructions, and sequenced (Macrogen, Seoul, South Korea).

Results and Discussion

For both *D. farinae* and *D. pteronyssinus* species, reverse transcription was performed on total RNA using either random hexamers or oligo-dT primer for the first strand synthesis. In both cases, PCR product of expected size 243 bp was obtained (Fig. 1, lines 2 and 3), suggesting that the NlpC/P60-like protein in house dust mites was of eukaryotic or mite origin. To check for a possible DNA contamination, PCR was performed directly from the RNA sample with negative result (Fig. 1, line 4). Product of the expected size (243 bp) was amplified from DNA of both *Dermatophagoides* species (Fig. 1, lines 5 and 6). We did not obtain any product from other tested mite species.

Sequence of the fragment from *D. farinae* showed 83% similarity to the previously described one in *D. pteronyssinus.* The BLAST search revealed no other eukaryotic homolog. Several homologous sequences were found in *Actinobacteria*, mostly described as invasins or their homologs (Fig. 2). The protein showed 54–56% identity with mycobacterial invasins or invasion-associated proteins and actinobacterial p60 homologs, and 53–55% identity with fungal NlpC/P60-like cell-wall peptidases. Although the closest homologs originated from the high GC actinobacteria, the fragment was distinguished by a low GC content, and the presence of 3-terminal poly(A) tail suggested its mite origin.

The gut of *D. farinae* Hughes 1961 is inhabited by a complex bacterial community (Hubert et al. 2012) including symbionts ("cadidatus *Cardinium hertegi*") (Kopecky et al. 2013). The endogenous enzymes such as NlpC/P60-like are suggested to regulate these bacteria.

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