IPD Project Details

Project ID: IPD9759

Project Title: From birth to bite: the evolutionary ecology of India's medically most important snake venoms

Description: Venoms of N. naja and D. russelii individuals of various developmental stages, including neonates (<30 days), juveniles (between 1 to 12 months), and mature individuals (>36 months), were sampled with permission from the state forest department of Karnataka. 226 individuals and 9 clutches were examined, including periodic venom collection (every three months) from the same N. naja and D. russelii juvenile individuals to track ontogenetic changes across time. Freshly collected venoms were flash-frozen in liquid nitrogen, lyophilised and stored at -80° C. Venoms were fractionated using RP-HPLC and SDS-PAGE and subjected to tandem mass spectrometry for toxin identification. The relative abundance of each toxin family were estimated and compared across different developmental stages.

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Sample Preparation: Venoms of N. naja and D. russelii individuals of various developmental stages were processed for MS/MS identification. The venoms were fractionated on a Shimadzu LC-20AD series HPLC system (Kyoto, Japan). A total of 1 mg of venoms were reconstituted in water and injected into a reversed-phase C18 Shim-Pack GIST column [4.6 x 250 mm, 5 ?m particle size and 100 Å pore size (Shimadzu, Japan)]. The fractions were eluted at a flow rate of 1 mL/min under following gradient of buffer A (0.1% TFA in HPLC grade water), buffer B (0.1% TFA in acetonitrile) : 5% B for 5 min, followed by 5–15% B for 10 min, 15–45% B for 60 min, and 45–70% B for 10 min and held at 70% r B for 9 min, followed by 70-98% B for 6 min and again held at 98% B for 5 min and completed with 5% B for 5 min. Fractions were collected separately by monitoring the absorbance peaks at 215 nm. A combined analysis strategy was adopted for mass spectrometry, wherein RP-HPLC fractions with higher peak area were subjected to reducing SDS-PAGE and smaller peaks that could not be visualised on the gel were directly subjected to in-solution digestion. The excised gel bands were destained using 30% acetonitrile in 50 mM ammonium bicarbonate buffer. The gel pieces were dehydrated using 100% acetonitrile, followed by reduction using 10 mM

dithiothreitol (DTT) at 56 °C for 45 min and alkylation using 55 mM iodoacetamide for 35 min at room temperature.

Peptide Separation: The samples were then digested using sequencing-grade trypsin and incubated overnight at 37 °C. The following day, the supernatant was collected and desalted in a Pierce C18 spin column (Thermofisher Scientific, USA) following the manufacturer's protocol before being subjected to mass spectrometric analyses. Similarly, for in-solution digestion, fractions were reduced and alkylated using DTT (100 mM) and iodoacetamide (100 mM), respectively, followed by tryptic digestion. The digested peptides were subjected to nano-Liquid Chromatography (nano-LC) and electrospray ionisation tandem mass spectrometry (ESI-MS/MS). The samples were injected into a PepMap C18 nano-LC column (50 cm x 75 ?m, 2 ?m particle size and 100 Å pore size) mounted on the Thermo EASY nLC Ultimate 3000 series system (Thermo Fisher Scientific, MA, USA), following a gradient elution of buffer A (0.1% formic acid in MS grade water) and buffer B (0.1% formic acid in 80% acetonitrile) at a constant flow rate of 250 nL/min for 90 min. An 8-35% gradient of buffer B was used over the first 70 min for elution, followed by 35 - 95% over the next 5 min, and finally 95% for the last 15 min. The following protocol was adopted to perform the MS scans: scan range (m/z) of 300 - 2000 with a resolution of 120000 and maximum injection time of 100 ms. To perform the precursor (MS) and fragment (MS/MS) scans, an orbitrap detector with high collision dissociation (HCD) fragmentation (30%) was used with the following parameters: scan range (m/z) of 110 –2000 and maximum injection time of 50 ms.

Protein Characterization: Raw MS/MS spectra were searched against the National Center for Biotechnology Information non-redundant (NCBI-NR) Serpentes database (taxid: 8570; with 549650 entries as of September 2023) to identify toxins that constitute the venom fractions. The search was performed in PEAKS Studio X Plus software (Bioinformatics Solutions Inc., ON, Canada) by setting parent and fragment mass error tolerance limits to 10 ppm and 0.6 Da, respectively. A 'monoisotopic' precursor ion search type and semispecific trypsin digestion were specified as parameters. Fixed and variable modifications were set as carbamidomethylation (+57.02) and oxidation (+14.99), respectively. The False Discovery Rate (FDR) was set to 0.1%, and PEAKS Studio automatically determined the corresponding -10lgP cutoff value. Hits with at least one unique matching peptide were considered for protein identification, and redundant hitsfrom each protein family were manually removed.

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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