The following supplement accompanies the article

Cell proteome variability of protistan mollusc parasite *Perkinsus olseni* among regions of the Spanish coast

Sergio Fernández-Boo, Antonio Villalba*, Asunción Cao

*Corresponding author: antonio.villalba.garcia@xunta.es

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Supplementary Materials and Methods

Sequencing of proteins by LC-MS/MS was performed as follows: samples were in-gel digested with trypsin (Promega, Madison, WI) using a Digest MSPro (Intavis, Koeln, Germany). Briefly, gel slices were washed with water and 20 mM ammonium bicarbonate pH 7.8, reduced with 10 mM DTT, alkylated with 55 mM iodoacetamide and digested with trypsin for 16 h at 37°C. Tryptic peptides were extracted with acetonitrile/water 0.25% TFA. Extracts were evaporated to dryness by vacuum and redisolved in 5 µl MeOH/H2O 2/1 0.1% TFA. MALDI-TOF spectra for each sample were obtained to ensure that the peptide contents were sufficient for successful LCMS analysis. Samples with detectable peptide concentrations were analysed by LC-MS/MS using a high resolution LTQ/Orbitrap mass spectrometer equipped with a microESI ion source (ThermoFisher, San Jose, CA). Each extract was diluted up to 40 µl with 1% formic acid. Samples were loaded in a chromatographic system consisting of a C18 preconcentration cartridge (Agilent Technologies, Santa Clara, CA) connected to a 10 cm long, 75 µm i.d. Vydac C18 column (Vydac, IL, USA). The separation was done at 0.4 µl/min in a 30 min acetonitrile gradient from 3 to 40% (solvent A: 0.1% formic acid, solvent B: acetonitrile 0.1% formic acid). The HPLC system was composed by an Agilent 1200 capillary nano pump, binary pump, a thermostated microinjector and a

micro switch valve. The Orbitrap instrument was operated in the positive ion mode with a spray voltage of 2 kV. The scan range of each full MS was m/z 400-2000. The spectrometric analysis was performed in an automatic dependent mode, acquiring a full scan and 5 MS/MS of the most abundant signals. Both MS and dependent MSMS scans were performed in the FT mode to maximise the mass resolution and precision of the MS spectra. A dynamic exclusion was set to 1 to avoid the redundant selection of precursor ions.

MS/MS spectra were used for searching protein sequences using SEQUEST (Proteome Discoverer, ThermoFisher, San Jose, CA) with the following parameters: peptide mass tolerance 10 ppm, fragment tolerance 100 ppm, enzyme set as trypsin and allowance up to two missed cleavages, static modification was cysteine carbamidomethylated (+57 Da) and dynamic modification was methionine oxidation (+16 Da). The Uniprot database (taxonomy: Alveolata) was used for searching. Identifications were filtered with Sf > 0.6, P(pep) < 0.005 and were manually validated. The chromatograms that gave negative identification with database search were reanalysed using the PEAKS *de novo* search engine tool (PEAKS Studio 5.2, Bioinformatics Solutions Inc., Waterloo, ON, Canada). The *de novo* search parameters were: mass tolerance 10 ppm for precursor ions and 100ppm for fragment ions. *De novo* sequences with an ALC score higher than 70% were sent to the Basic Local Alignment Search Tool (BLAST) for protein identification. Uniprot database (taxonomy Alvelolata) and SwissProt database (taxonomy: mammals + human) were used in BLAST. The mammals+human database was used in order to detect sequence tags related to probable keratin and trypsin contaminations. These sequence tags were rejected.