Semi-purified antimicrobial proteins from oyster hemolymph inhibit pneumococcal infection

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SUPPLEMENTARY FIGURES

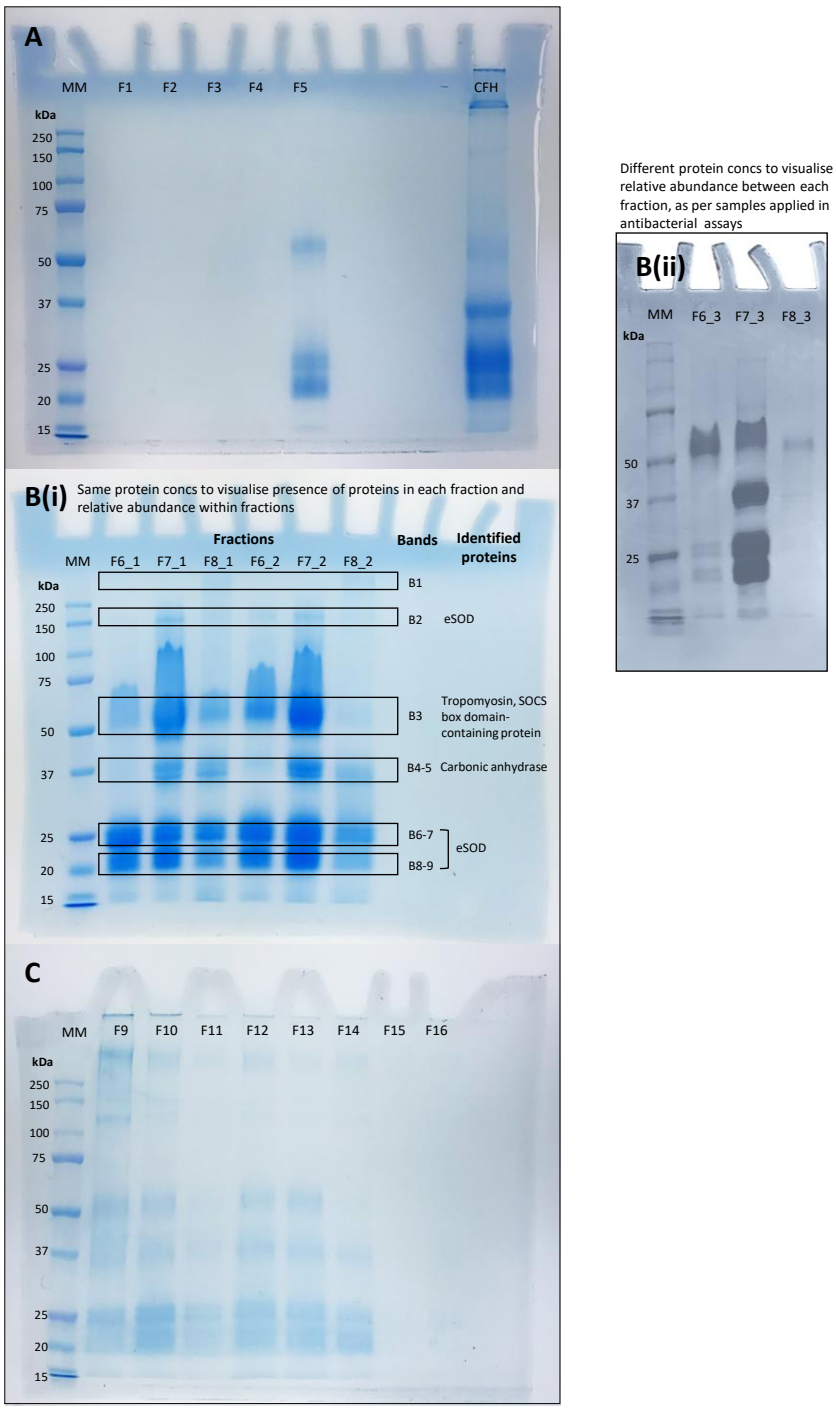

Figure S1: SDS-PAGE gel runs of 16 fractions of SRO hemolymph. Figure Bi= Fraction 6, 7 and 8 from two biological replicates of 6-8 µL representing 1-2 µg total protein in Laemmli buffer. CFH= cell-free hemolymph. Figures A and C= one biological replicate of ~20 µL representing much lower protein concentrations. Figure Bii inset= Fraction 6, 7 and 8 showing relative abundance when all samples added 10 µL at 25% concentration in buffer. Molecular marker (MM) was positioned in the first well of each gel and buffer was added to empty wells. Proteins in each band were identified by HPLC-MS/MS; proteins with high relative abundance in Fraction 7 were likely responsible antimicrobial for activity. Multiple bands corresponded to the same protein for various reasons (protein isoforms, degradation, complexes, etc.). Some proteins (e.g. cofilin, gelsolin-like protein 2, cystatin) were not visible or detected due to having lower abundance than dominant proteins (e.g. carbonic anhydrase, extracellular superoxide dismutase [eSOD], tropomyosin) but may still contribute to overall activity.

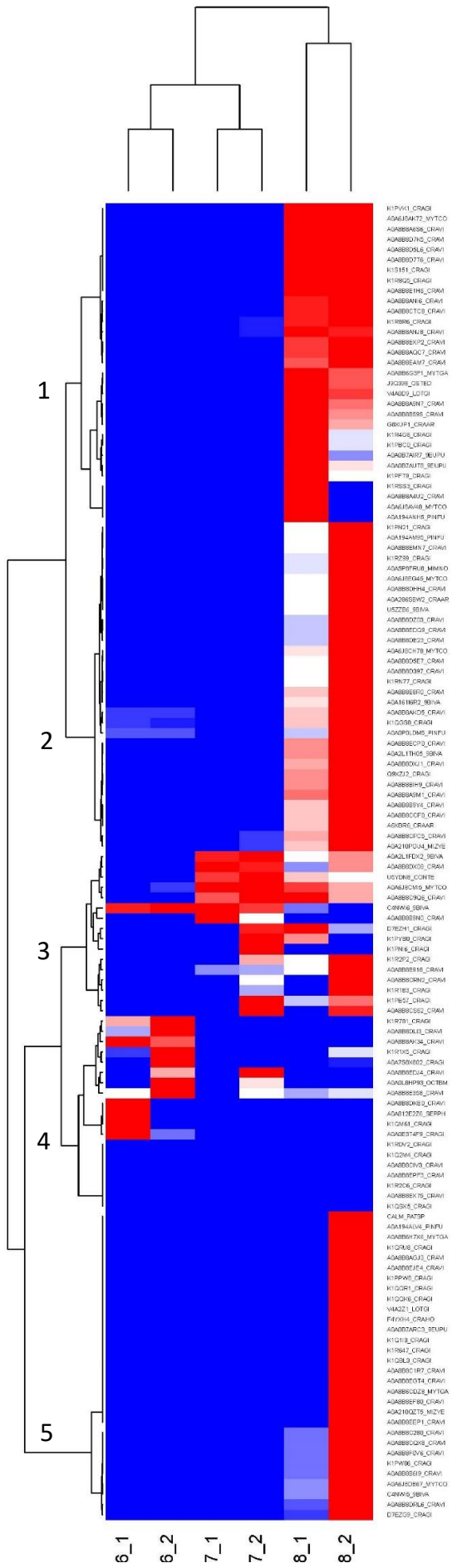

Figure S2: Hierarchical clustering (Ward's method) heat map of identified proteins with annotations correlating to data supplied as supplementary in Fractions 6, 7 and 8 from two biological replicates of hemolymph from the Sydney Rock Oyster, *Saccostrea glomerata* with numbered groupings based on scaled abundance in respective fractions.

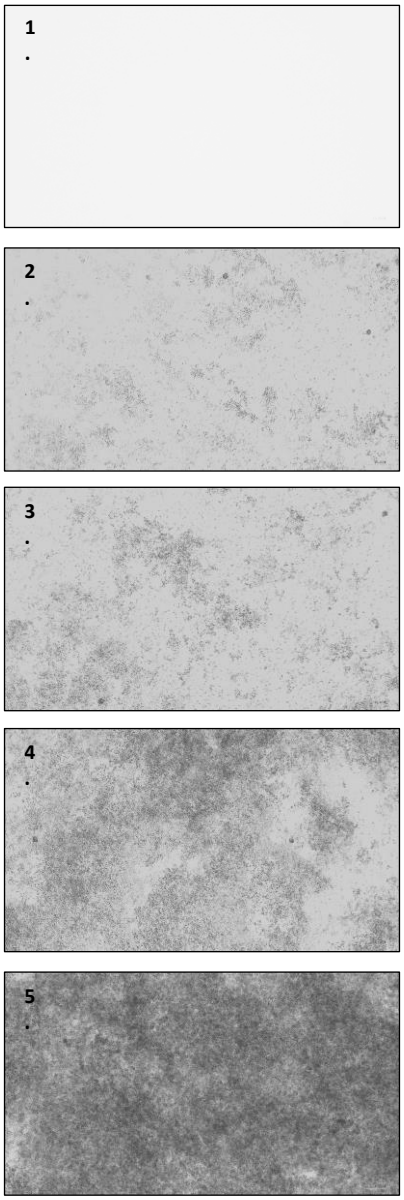

Figure S3: Images of *S. pneumoniae* cells showing the antimicrobial activity of Fraction 7 at 1) 150 μg/mL, 2) 75 μg/mL, 3) 37 μg/mL, 4) 15 μg/mL protein, compared to 5) the positive growth control, taken at 20x magnification using a Nikon Eclipse Ts2 inverted microscope.

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BovCA   3    SPDWGYDGENGPEHWGKLYPIANGNNQSPIDI-KTSETKRDPSLKPLSVS-YNPATAKEI  60
             + DW Y GE+G   W   YP      QSPID  KT +   D  L     + ++  +   +
SROCA   16   AADWHYTGEHGTSDWPHEYPDCGLVRQSPIDFPKTEDMTYDSKLTQFQFTGFDDLSRYSL  75

BovCA   61   V--NVGHSFHVNFEDSDNRSVLKGGPLSESYRLRQFHFHWGITDDCGSEHLVDGAKFSAE  118
             V  N GH+  +     D   +++GG L   ++  QFHFHWG +D+ GSEH  DG  F  E
SROCA   76   VLHNNGHTAVIKVTGGD--LLVEGGGLPGRFKTAQFHFHWGHSDNEGSEHTFDGHSFPLE  133

BovCA   119  LHLVHWNSAKYPSFADAASQ-ADGLALIGVLVKVGQAN-PNLQKVLDALKAVKNKNKKAP  176
             LH+V++N  KY S ADAAS+  DGLA++G   +V   N  ++  +++ L  V  K    P
SROCA   134  LHIVNYNE-KYGSLADAASKDLDGLAVLGFWYEVSHNNNDDIAPLIEQLSHVPTKGSSVP  192

BovCA   177  FTNFDPSVLLP-----PSLDYWAYSGSLTHPPLHESVTWIIFKETISVSSEQLAQFRSLL  231
              T F+ + LLP         ++ Y GSLT PP  +SV W +F++TI +SS+QL+ FR+L
SROCA   193  LTGFNLAQLLPIHNIQSKSHFFRYPGSLTTPPCFQSVVWTMFQQTIPISSQQLSMFRALH  252

BovCA   232  ANAEGDREVHIKQNNRPPQPLNGRTVKASF  261
              + E   + ++  N RP QPLNGR +  +F
SROCA   253  EDQELQSDHYLVDNFRPIQPLNGRVIYRNF  282
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Figure S4: BLAST alignments of protein sequences for commercial carbonic anhydrase from bovine erythrocytes (BovCA) and SRO hemolymph carbonic anhydrase (SROCA). Identities: 101/270 (37%), Positives: 148/270 (54%), Gaps: 14/270 (5%).