

Evolution of Th2 responses: characterization of IL-4/13 in sea bass (*Dicentrarchus labrax* L.) and studies of expression and biological activity

Valentina Stocchi^{a1}, Tiehui Wang^{a2}, Elisa Randelli¹, Massimo Mazzini¹, Marco Gerdol³, Alberto Pallavicini³, Chris J. Secombes², Giuseppe Scapigliati¹, Francesco Buonocore^{1*}

¹Department for Innovation in Biological, Agro-food and Forest systems, University of Tuscia, Largo dell'Università snc, 05100 Viterbo (VT), Italy

²Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK

³Department of Life Sciences, University of Trieste, Via Giorgieri 5, 34127 Trieste (TS), Italy

^aThese authors contributed equally to the paper

*Corresponding author at: Department for Innovation in Biological, Agro-food and Forest systems, University of Tuscia, Largo dell'Università snc, 05100 Viterbo (VT), Italy, E-mail address: fbuono@unitus.it

Signal peptide

1 M K M G M L L L V S A L
1 AAGGACTTCTTGAAATAGCTTCTTCCAGAAGTTGACAGCCAAACAACTCTGATCATGAAGATGGGGATGCTTCTGCTGGTTTCAGCTCTG

13 V L L S A V S P T I A S P S P H H H K N L N I V F D M A Q K
92 GTGCTGCTCTCTGCCGTGAGTCCCACCATCGCCAGTCCTTCTCCTCATCATCACAAGAACCCTAAACATCGTATTTGACATGGCTCAAAAA

43 Y N E S L S R M Y F V E D V S S L A D G A N K C Q D K F F C
182 TACAACGAATCTCTCTCTCGGATGTACTTTGTGGAGGACGTGTGAGTCTGGCTGATGGTGCAAACAAATGTGAGGATAAGTCTTCTGCT

73 K V Y M I L R E H E E L I N R S E E R G L V K N L K K F V D
272 AAAGTGTACATGATCCTGCGTGAACACGAAGAATTGATTAACCGATCTGAGGAGCGTGGCCTGTGAAGAACCTGAAGAAATTTGTTGAT

103 G I N A N C T E L L K D V V P S D V T K P I P S L L E H L T
362 GGCATAAATGCAAACGTACGGAGTTACTAAAGGATGTGGTCCCTTCAGACGTCACAAAACCGATACCCTCCCTTTTAGAACACCTCACC

133 R C I Q S L N M R R S D -
452 CGCTGTATCCAGAGCTTGAACATGAGACGAAGCGATTAATGAACAGACCTGCGACCCGACTGTGAGTGGAGTCCCTTGAGAGTTTCATTTT

542 GTTTTTTGAGAGAATTTTATTTTTTACATTTTGA AAAA CTGTAATGTAATGACTAATAGCAAATTAATGGGGAATATGATTAGATAATA
632 TGTTGTATACCAGGTTAAATGTAAATTTAATTTTTTCTACGTAATGTGCTTGATAGTTTTTATTTAAGGAGTTATCTTCAAGTGAATT
722 AATATCTGAAGTCCCTCTAGACGATGCACAGCCGTATAGTTTTGTACTACAACAACAACAATGTAATAAATAGTCATTTAACAGAT
812 ATTTACTTTTATCTGCCCCGTAAGCAGTTTTGAATTAGTACAGTGTGATGATCACTGTGGTGTGCTGAGCACTATCAAATACACCTTCATAT
902 TTATTCTTTTTCAGTATTTAACATAAGTTAAGGCTGTTTTATATATTTGAGTGGCATATTTATTGTAATCCTATCATAACAGCATTGAAATA
992 TGTGTTTACAGAAAAATAAATCGTATTACCCATGAGTGGAGTATATATGGAAATGCTTTGTGCTCACATGTTAATCAGAATTTACAGAAA
1082 GACTTTTTATAACTTTTATTTTTATCTGTTGCATTGATTTTCTTTATGATGCTGTGCTTTTTATCAAGTCATCTTTTCTCATTGAGTCTC
1172 ATTCAACTACTCACACACCTCTAAATATGTTAAGTTACATTTAAACTGCAGATTATGAGTGCAGCTGAACACAAATTTAACCTGTATCC
1262 ATTTTTTTTTGTTTCACTTTTCATTTCTTTTAAATACATTATATTTGTAGCATTGACCTCATTGCATACTCATTGGCATTAGAATATCTT
1352 TGGTACTCTCAAATTTAATTAGACATCAGTTATTTAGAGATGGATCAAACCAAGATGTTTGTAAATGGATCTAGTTAAAGCACTGCAAAA
1442 CGCAAAAATAATTCAATATAAGAAGGCCAAATGACTCTGAGTTTGTAGAGCACTGGCTGTGATTTGTTTGGATCATGTCCTTTTCTGTAAGA
1532 CTAGTGGGGAACCACACAATAAAGTATTGGATTTATGTATATCTTTGACTTGCCTTGGACCCAAATTTGTACAAGTGTTTAAACGGCCT
1622 GTTTCTGTTGTAGTTTGCCTGCTACAACAATAAATGACTATATTGTCAAATCTTTGTATGGATAGATGTTGCCGTGTTCTTTACCCTAG
1712 AGACAGATTGTAACATTCATATTAAAATTTAATTTATTTCAGCTTTTGTATCATGATCCACGT

Figure S1. The nucleotide sequence and amino acid translation of sea bass IL-4/13A1. Start and stop codons, and in frame stop codon before the main ORF are underlined; ATTTA motifs are in bold and underlined, potential poly(A) signals are boxed; potential N-glycosylation sites are underlined.

CGTCTCATCAACCACACAATAATCAGTCATATCTTATCTTACATTACCGACTGACAGCATTTTTTTCCTTTTTGGTATGAGTGGTAAAGC

92 TTGGCCGAGGCGTGGGCAATGCAAAAGTATAAAGTTCTCAGTTGAAGCCTTATAACAACTGATCAATTCAAGGGCTTTCTGACATAGC

Signal peptide

1 M K M K M L L L V S A V A L L V N S A A

182 TTCCTCAAGAGCTCAACAACAGCTTGTATCATGAAGATGAAGATGCTTCTGCTGGTATCTGCTGTGGCTCTGCTGGTAAATTCAGCTGCT

21 V S A R P H N V T Q Q N L I F D L V E K C I E S R S Q T F V

272 GTTAGTGCTCGTCCCTCATAACGTTACTCAGCAAAACCTAATATTTGACCTCGTGAAAAATGCATTGAATCTCGTTCACAGACATTTGTG

51 D D V S H L A K G S R K C E D R F F C K V H D V L R N T K D

362 GATGATGTATCACATCTGGCTAAAGGCAGCAGAAAATGTGAGGATCGGTTCTTTTGTAAAGTGCATGATGTCTGCGTAACACAAAAGAT

81 V C K E E D R K V L V E T L H A Y N T G R N V Q C E N T L Q

452 GTCTGTAAAGAGGAGGACAGGAAGGTGCTTGTGAAACCCTGCATGCGTATAATACTGGCAGAAATGTGCAGTGTGAAAATACACTTCAG

111 G M T S T G I E I E V S S F L E H V K R C V R H R N F H G T

542 GGAATGACAAGTACAGGCATAGAGATCGAAGTATCCAGCTTTTTGGAACATGTCAAGCGTTGTGTCGGGCACAGAACTTTCATGGGACC

141 K K -

632 AAAAAATAGCAGCTCCTGAATACCACAAAACCAATGTGAAGGCTACCAGATACAGCTGCTTGTTTTCTAATTTGTAAGTGT**ATTTAAC**

722 ATAAATTATTAATCTATTTGAATAACAT**ATTTA**TTGTAATATCATGCAGCATTGAAATACAATTGTACAGAA**ATTTA**CAGAAA**AATAAAA**

812 TTGTATTCATTAAGATAAAAAAAAAAAGT

Figure S2. The nucleotide sequence and amino acid translation of sea bass IL-4/13A2. Start and stop codons, and in frame stop codon before the main ORF are underlined; ATTTA motifs are in bold and underlined, the potential poly(A) signal is boxed; potential N-glycosylation sites are underlined.

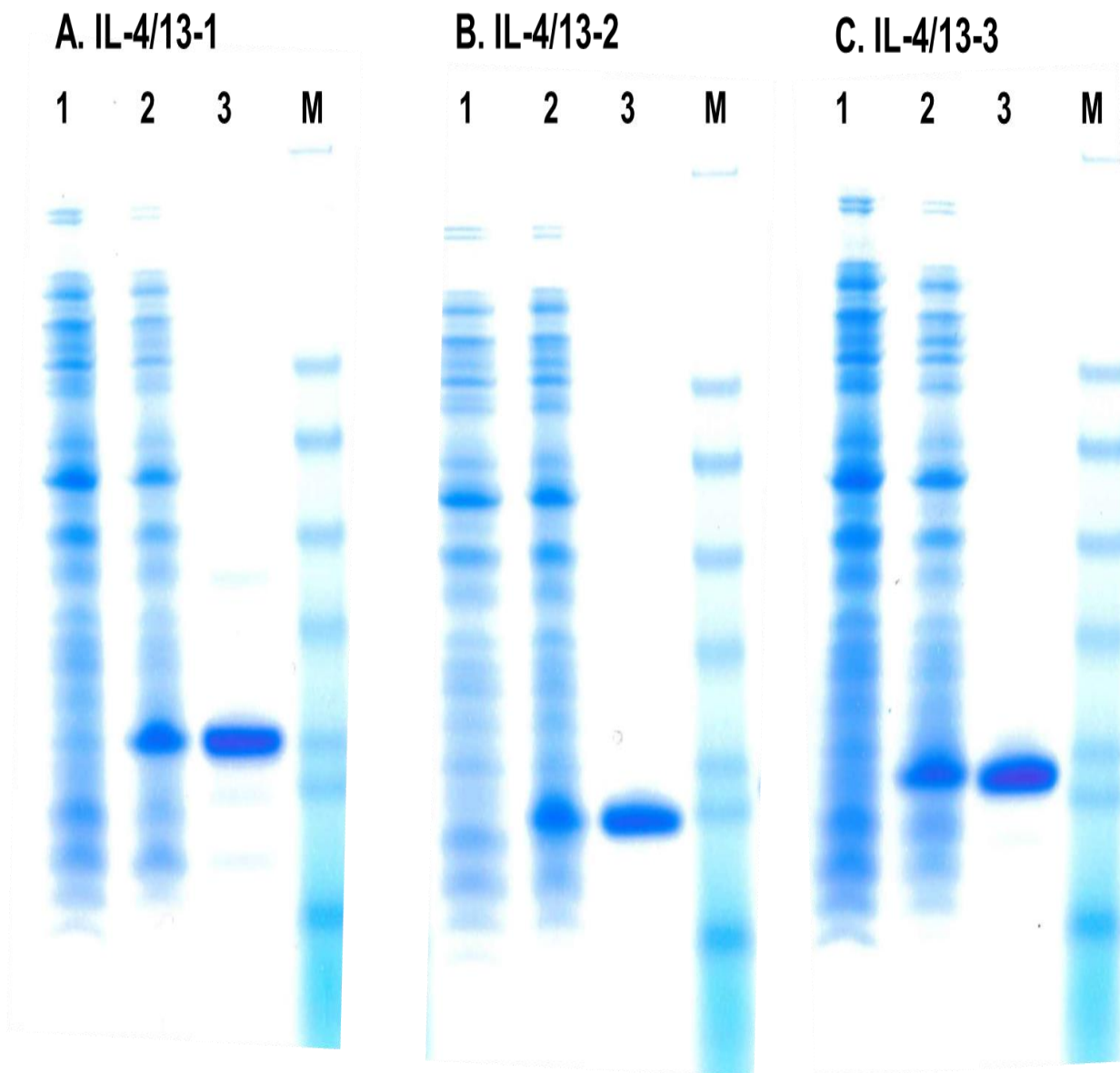


Figure S4. SDS-PAGE analysis of seabass rIL-4/13 isoforms expression and purification from *E. coli* BL21 Star (DE3). The SDS-PAGE gel was stained with SeeBlue (Invitrogen). Samples loaded in the different lanes: 1: a sample from un-induced BL21 cells; 2: BL21 transformed by IL-4/13-expressing plasmid and induced with 1 mM IPTG for 4 h; 3: expression product purified from transformed cells expressing IL-4/13 isoforms; M: Protein marker, SeeBlue (Invitrogen); IL-4/13-1 = IL-4/13B; IL-4/13-2 = IL-4/13-A1; IL-4/13-3 = IL-4/13A2.