# Molecular identification and expression analysis of bactericidal permeability-increasing protein/ LPS-binding protein (BPI/LBP) from Black rockfish *Sebastes schlegeli*

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Bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP) are important components of the mammalian innate defence system against Gram-negative infections. The BPI/LBP cDNA was identified from the black rockfish ConA/PMA or LPS stimulated leukocyte cDNA library. The full-length BR-BPI/LBP cDNA was 2118 bp long and contained an open reading frame (ORF) of 1422 bp that encoded 473 amino-acid residues. The 5' UTR had a length of 57 bp, and the 3' UTR 639 bp. The molecular weight and theoretical isoelectric point (p*I*) values were calculated 51.4 kDa and 9.72, respectively. Compared with other known BPI or BPI/LBP peptide sequences, the most conserved regions of the black rockfish BPI/LBP peptide were found to be the BPI1 N-terminal, BPI2 C-terminal domains and a LPS binding domain. Phylogenetic analysis based on the deduced amino acid sequence revealed a homologous relationship between the BPI/LBP sequence of black rockfish and that of other teleosts. The black rockfish BPI/LBP gene was predominantly expressed in the PBLs, head kidney, trunk kidney and spleen. The expression of the black rockfish BPI/LBP molecule was induced in the peripheral blood leukocytes (PBLs) from 1 to 24 h following LPS stimulation, with a peak at 12 h post-stimulation.

Key words Black rockfish, BPI/LBP, LPS stimulation, Real-time PCR

There are two types of immunity, innate immunity and adaptive immunity, which have different roles against the infection of pathogens (Dempsey *et al.*, 2003). The innate immune system has the potential for quick, powerful, and non-specific responses to a wide range of pathogens (Xu *et al.*, 2005). Moreover, innate immune system is a varied and complex group of phenomena and has a role as a first line of defense

†Corresponding Author: Chan-II Park E-mail: vinus96@hanmail.net TEL: 82-55-640-3103, FAX: 82-55-642-4509 against infections (Kono and Sakai, 2003). The host innate immune system recognises invading pathogens by their conserved pathogen associated molecular patterns (PAMPs) using pattern recognition receptors (PRRs) (Medzhitov and Janeway, 2002). Lipopolysaccharide (LPS), a complex glycolipid and one of the major components of the outer surface membrane of Gram-negative bacteria, is one of the PAMPs. LPS plays an important role in the activation of innate immunity (Alexander and Rietschel, 2001).

Two of the proteins crucial to the mediation of signals for LPS are bactericidal permeability-increasing protein (BPI) and LPS-binding protein (LBP), which share several characteristics. Both belong to a family of lipid transfer/LPS-binding proteins (LT/LBP), which also include the mammalian phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) (Bingle and Craven, 2004). They are composed of an N-terminal domain that binds to LPS and a C-terminal domain that mediates the delivery of LPS to other host molecules involved in the response to LPS (Schumann et al., 1990; Iovine et al., 1997; Beamer et al., 1998). Even though these two proteins have similar structures, they are considered to have antagonistic functions. LBP binds LPS and enhances cellular responses to LPS, while BPI binds LPS but reduces cellular responses (Tobias et al., 1988; Tobias et al., 1997).

The complete cDNA sequences of BPI and LBP have been cloned from human (Gray et al., 1989), bovine (Leong and Camerato, 1990), rabbit (Zarember et al., 1997; Schumann et al., 1990), mouse (Gallay et al., 1993; Lengacher et al., 1995) and rat (Su et al., 1994). The BPI or LBP homologues of teleosts have not been classified as either a BPI or a LBP because the functional properties of these proteins have not been characterized. Therefore, in teleosts, they have been referred to as BPI/LBP (Nam et al., 2010). The BPI/LBP genes have been isolated and characterized in some fish species. In particular, the BPI/LBP gene was sequenced from rainbow trout (Inagawa et al., 2002), Atlantic cod (Stenvik et al., 2004), common carp (Kono and Sakai, 2003), channel catfish (Xu et al., 2005), olive flounder (Nam et al., 2010), and ayu (Suzuki et al., 2009).

Furthermore, a cDNA sequence with homologies to members of the LBP/BPI family was identified in the oyster *Crassostrea gigas*, and this invertebrate LBP/BPI family is the first to have been characterized by functional analysis using a BPI recombinant protein (Gonzalez *et al.*, 2007). The presence of the BPI/LBP gene in black rockfish, however, has not been reported to date.

Black rockfish (*Sebastes schlegeli*) is one of the more significant fish species in Korea due to the human interest in aquaculture and fisheries, but diseases, especially infectious ones, have occurred frequently in them and have limited the production efficiencies of this economic fish. To improve the black rockfish production efficiency, it is necessary to understand the cellular and molecular processes involved in the disease resistance to this species. In this paper, the cloning, characterization, and expression analysis of the BPI/LBP gene from black rockfish (*Sebastes schlegeli*) are reported.

# Materials and Methods

#### Identification of Black rockfish BPI/LBP cDNA

Black rockfish BPI/LBP full-length cDNA was identified via the EST analysis of the ConA/PMA or LPS stimulated PBLs library (Baeck *et al.*, 2008). 5'-termini base sequencing of the selected cDNA clones in phagemid form was performed using an ABI 3100 automatic DNA sequencer (PE Applied Biosystems, CA, USA) and an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, USA). Bioinformatic analysis was conducted to determine the gene identities, using the Genetyx ver. 8.0 software (SDC Software, Japan). Briefly, the vector sequences were removed, and the database search was limited to the ESTs that are >400 bp in length.

Multiple alignment and phylogenetic analysis

The sequence of BR-BPI/LBP cDNA was compared with the sequences in the peptide sequence databases at the National Center for Biotechnology Information (NCBI), using the BLAST network service. Nucleotide sequence comparisons were carried out using the BLASTX program (Gish and David, 1993).

The multiple alignment of the amino acid sequence of the BR-BPI/LBP proteins was performed using the Clustal W program (Thompson et al., 1994). The BPI or BPI/LBP sequences of that were used for the comparison and their GenBank accession numbers were as follows: Cattle BPI (NP 776320), Channel catfish BPI (AAX20011), Dog BPI (XP 534417), House mouse BPI (NP 808518), Human BPI (ABD66755), Norway rat BPI (AAH79318), Pig BPI (ABO34136), Atlantic cod BPI/LBP-A (AAM52335), Atlantic cod BPI/LBP-B (AAM52336), Avu BPI/LBP (BAG49475), Common carp BPI/LBP (BAC56095), Olive flounder LBP/BPI (ACV74252), Rainbow trout LBP/BPI-1 (NP 001118057), and Rainbow trout LBP/BPI-2 (NP 001117670). The primary structure of the deduced amino acid sequence of BR-BPI/LBP was analyzed by ProtParam (http ://cn.expasy.org/tolls/protparam). The positions of signal peptide and BPI domains was predicated with the Simple Modular Architecture Tool (SMART) (http://smart. embl-heidelberg.de/).

The phylogenetic relationships were inferred using the Mega 4 program and distance analysis through the neighbor-joining method. The accession numbers of BPI or BPI/LBP for the creation of phylogenetic tree used are described above. In addition to accession number of LBP amino sequences are as follows: Cattle LBP (NP\_001033763), Dog LBP (XP\_542993), House mouse LBP (NP\_032515), Human LBP (AAB31143), Norway rat LBP (NP\_058904), Pig LBP (NP\_001121907). The values supporting each node were derived from 2,000 re-samplings.

# RT-PCR analysis of the BR-BPI/LBP transcripts in different tissues

Eight tissues (PBLs, head kidney, trunk kidney, spleen, liver, intestine, gill, and muscle) were isolated from approximately 350 g healthy black rockfish. The total RNA from each tissue was extracted using TRIzol reagent (Invitrogen, USA), according to the manufacturer's instructions. First-strand cDNA synthesis was carried out using a first-strand cDNA synthesis kit (Takara, Japan), according to the manufacturer's instructions. The first-strand cDNAs were used as a PCR amplification template, with the specific primers. The oligonucleotide primers used to amplify the BR-BPI/LBP cDNA fragment were BrLPS-F and BrLPS-R. Thermal cycling was performed using TaKaRa PCR Thermal Cycler (Takara, Japan) with ExPrime Taq premix (GENET BIO, Korea) as follows: predenaturation at 94°C for 5 min, 25 cycles of denaturation at 94  $^{\circ}$ C for 30 s, annealing at 55  $^{\circ}$ C for 1 min, extension at  $72^{\circ}$ C for 1 min, and final extension at 72 °C for 7 min. The expression of  $\beta$ -actin was used as the internal control. The PCR amplification products were observed on 1.5% agarose gel electrophoresis with a 100-bp DNA ladder (Takara, Japan). The primer

Primer name	Sequence(5'-3')	Usage	Amplify (bp)
B1LPS-F	CTATTGCCATCAAGAGTGATG	RT-PCR	460
BrLPS-R	GTGTTGTAGACGAAGGCTGCA		
BrLBP-F-2	GTGTCTCCCATTGGCAAAGT	Real-time PCR	150
BrLBP-R-2	CCTCCAGTTTCCGTGCATAC		
β-actin-F	TTTCCCTCCATTGTTGGTCG	RT-PCR, Real-time PCR	200
β-actin-R	GCGACTCTCAGCTCGTTGTA		

Table 1. Primers used for all the experiments

sequences used in this study are listed in Table 1.

Real-time PCR analysis

Black rockfish (300-350 g) was purchased from the Fisheries market in Tong-yeong, Korea. Peripheral blood samples were collected in a heparinized (100 Ul) syringe from the caudal blood vein. Peripheral blood leukocytes (PBLs) isolation and PBLs stimulation with LPS (50  $\mu$ g/ml) were carried out as a previously reported method (Beack et al., 2008). The total RNA was extracted, and first-strand cDNA synthesis was performed as described above. Real-time PCR was performed on a Thermal cycler Dice real time system (Takara, Japan) with SYBR Green I (Takara, Japan). The threshold cycle (Ct) values were automatically calculated as follows: the cycle when the fluorescence of the sample exceeded a threshold level corresponding to 10 standard deviations of the mean of the baseline fluorescence. Amplification was performed 3 step PCR methods as follows: 1 cycle at  $95^{\circ}$  for 10 s and 45 cycles at  $95^{\circ}$  for 30 s,  $62^{\circ}$ for 30 s and 72  $^\circ\!\mathrm{C}$  for 30 s, with a dissociation step at 95  $^{\circ}$ C for 15 s, 62  $^{\circ}$ C for 30 s and 95  $^{\circ}$ C for 15 s. B-actin was detected as an internal control. All the samples were analyzed in triplicate and the expression of target genes was calculated as relative folds of the expression of  $\beta$ -actin according to the  $2^{-\Delta\Delta CT}$  methods.

## Results and discussion

In the present study, we report for the cloning and characteristics of the LBP/BPI gene from the black rockfish, Sebastes schlegeli. The full-length BPI/LBP cDNA was 2118 bp long and contained an open reading frame (ORF) of 1422 bp that encoded 473 amino acid residues. The molecular mass were calculated 51.4 kDa and theoretical isoelectric point (pI) values were calculated 9.72. The 5' UTR was 57 bp in length, and the 3' UTR 639 bp, and they contain a polyadenylation signal (AATAA) and a polyadenylation site (accession number: AB548677) (Fig. 1). These sequences had a putative 18 AA signal peptide, followed by 455 AA residues namely identified as putative mature proteins. Mammalian BPI or LBP family proteins have signal peptide. This suggests that these proteins could be secreted in the mature form after cleavage.

The Black rockfish BPI/LBP has a BPI/LB P/CETP-specific sequence motifs BPI1 (BPI/LBP/CETP N-terminal domain) between amino acid residues 8 and

1	GTTTGCAGTCCGACTGACACATCAAGAAGAACCTGCAGAAAACTGCTGCTCCGGTCAAGATGGTCCTGTGCTGTGGCTGGC	90 -11
91	TTAATCCCTATGACCTTAAGCATCAATCCTGGGGTGAAGGTCAGGCTAACAGGAAAAGGCCTTGAATATGGCAGACAACTGGGGATGGCT LIPMTLS $_1$ NPGVKV <u>RLTGKGLEYGRQCLGMA</u>	180 23
181	TCCATCCAGCAGAAACTCAAAACCATCAAAGTCCCAGATATATCAGGGAAACAAAGG <u>GTGTCTCCCATTGGCAAAGTC</u> AAGTACAGCCTG <u>S I Q Q K L K T I K V P D I S G K Q R V S P I G K V K Y S L</u>	270 53
271	BrLBP-F-2 primer         TCAAATATGCAAATAGTGGACGTGGGATTGCCAAAGTCTGGCGCGGATCTGGTGCGAGGGACTGGTGTCAAACTGTCCATTGGTAACGCC         S       N       M       Q       I       V       D       L       V       P       G       T       G       N       A	360 83
361	LPS binding domain TTCCTCCGTATGCACGGAAACTGGAGGGGTCAAGTACCTCCGAATAATAAAGGACAGTGGCTCTTTTGATTTGAATGTCAATGATCTCACT F L R M H G N W R V K Y L R I I K D S G S F D L N V N D L T	450 113
451	BrLBP-R-2 primer ATCACAACAACTATTGCCATCAAGAGTGATGAGACAGGCCGACCTGTGGGTCAGCAGTGTCAACTGTGCGGCCACTGTCGGCAGTGCGAAG I T T T I A I K S D E T G R P V V S S V N C A A T V G S A K	540 143
541	$\begin{array}{cccc} \textbf{BrLPS-Fprimer} \\ \textbf{ATCAAAATTCCACGGTGGAGCCAGCTGGCTGTACAATCTCTTCAGAAAGTTCGTTGATAAGGCTATACGAAATGCACTGCAGAAACAGATG} \\ \underline{I \ K \ F \ H \ G \ G \ A \ S \ W \ L \ Y \ N \ L \ F \ R \ K \ F \ V \ D \ K \ A \ I \ R \ N \ A \ L \ Q \ K \ Q \ M \end{array}$	630 173
631	TGCCCTCTGGTGGCCAACGCAGTATCTGATCTGAACCCTCAGTTGAAAACTCTGAATGTTTTAGCCAAGGTGGACCAGTATGCAGAGATT _C P L V A N A V S D L N P Q L K T L N V L A K V D Q Y A E I	720 203
721	GAATATTCCATGGTGTCATCGCCCACAGTGTCAAACTCTTGTATTGACTTCAGCTTGAAGGGTGAATTTTACAACATCGGGAAGCATCAG	810 233
811	GAGCCTCCGTTCTCCCCGCAGCCTTCTCCCTGCCGCCCCAGATCAACATGTTGTATATCAGCGTGTCTGCCTTCACCATCAACTCT	900 263
901	Central domain <u>GCAGCCTTTGTCTACAACAC</u> AGCTGGAGCCCTCGAGCCTGTACATCACTGATGACATGATCCCCACAAGCATCTCCCCATCCGACTCAACACC <u>A         F         V         Y         N         T         A         G         A         L         Y         I         T         D         M         I         P         O         A         S         P         I         R         L         N         T  </u>	990 293
991	BrLPS-R primer AGAACGTTTGGAGCCTTCATCCCGCAGGTTGCCAAGCGTTTCCCAGGACTGATGATGATAGATGCTGGTGAAGACGGCGAAAAACCCTGTC R T F G A F I P Q V A K R F P G L M M K L L V K T A K N P V	1080 323
1081	GTCACTTTTGAACCCAACAAAGTGACAGTTCAGGCCACTAGCACAGTGACGGCCTACGCTATCCAACCCAACACCACGCTTTCACCGCTC	1170 353
1171	TTTGTCCTAAACTTGGAGACGAGTGTCAGCGCCCGAGTGTTTGTCAGTGGAATGAGGCTGGCCGGAGCTGTCACCCTAAACAAAATGGCT <u>F V L N L E T S V S A R V F V S G M R L A G A V T L N K M A</u>	1260 383
1261	CTGACCCTGGGGACAAGTTATGTGGGAGAGTTTCAGGTCAGATCCCTCGACAGCATCTTCCAAGTGGTCCTCAAAGTTGTTGTGATACCT L T L G T S Y V G E F Q V R S L D S I F Q V V L K V V V I P	1350 413
1351	ATACTGAACGTTCAACTTGCGAAGGGATATCCACTTCCTACACTTGGAAGATGAACCTCTTGAACACTGAGCTTCAGGTCCTGAAGGAC	1440 443
1441	TATATGCTGATTGGGACGGATGTTCAGTTCACGGGTTGAATGGCATTCTGGAAACTCCCAGGATTCATTTCTGCATTGGGGCATGCAAC <u>Y M L I G T</u> D V Q F T G $\star$	1530 455
1531 1621 1711 1801 1891 1981 2071	CATGAAGGCAATCACAACCAGAGTTCAACTACCTGACGGACAGATTTCAAAGAACAAACTGAATGATGCACAAAATATTGTTTCTACGAGA AATGCATATTCCTGTTTGTACTTAGATATCACATGAGGTTTTTATAAATTTTTCTGTTCTTTATGCTATTTTCTTTTTTTT	1620 1710 1800 1890 1980 2070

Fig. 1. Nucleotide and deduced amino acid sequences of black rockfish BPI/LBP. The oligonucleotide primers that were used in the study are indicated with arrows. The putative signal peptide is indicated black box. Central domain and LPS binding domain is indicated box and gray. N-terminal domain and C-terminal domain was indicated underline and double underline, respectively. The polyadenylation signal (AAUAA) and poly A tails is indicated in boldface and underline.

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Black rockfish	1:MVLCCWLALVALI-PMTLSINPGVKVRLTGKGLEYGRO	GMASIOOKLKTIKVPDISGKORVSPIGKVKYSLSNMOI 76
Olive flounder	1:MSLHCQLILVLFI-AVASATNPGVKVKLTEKGLEYGRQ	GMASIQQKLKTIKVPDISGKERVPPIGKVEYSLSHMQI 76 AVASLOKKLMTIKVPDISGKERVKPIGKVRYSVTEMRV 76
Atlantic cod A Atlantic cod B	1:MFEWRWLALLALI-PLALSANAGVKVKLTQKGLDFGRE 1:MFEWRWLALLALI-PLALSANAGVKVKLTQKGLDFGRE	AVASLOKKLMTIKVPDISGKERVKPIGKVRISVTEMRV 76
Ayu	1 · MSAWLTLLALT_ DLTLATNDCVKVKLTEKCLEVCDE	CMARLORVIKETKUPDIRGRAKUADIGKUTVRIEGMOT 74
Rainbow trout 1	1:MSFCCWLALLALV-FFALATNPGVKVKLTEKGIEYGKQ 1:MSFCCLLALLAVI-STALATNPGVKVKLTEKGIEYGKQ	IGMASLQQKLKTMKVPDLSGTERVAPIGKVKYSLTGITI 76
Rainbow trout 2 Common carp	1:MSPCCLLALLAVI-SLTLAASPGVKVKLTDKGIEYGKQ	IGMASLQQKLKTMKVPDLSGTEKVPPIGKVKYSLTGMTI 76 IGIASIOOKLRTIKVPDISGTEKVDPIGKVOYSFTGMOI 76
Common carp Channel catfish	1:MFLWCVLVLLNLV-SVATGTNAGVKVRLTQKGLEYGRQ 1:MVSLWCVLALLSFFSPEASGTNFGVILRVTQKGLEYGRQ	IGLASIQQKLKTIKVPDISGTEKVDPIGKVQYSFTGMQI 76 IGLVTLQKKLKTVKIPDMSGSEKVSPIGKVSYSLTGIQI 78
Norway rat	1:MAWGPDNVRKWSSLALLAIVGTA-LTAATDPGFVARISOKGLDFVCO	SMVELQKELLAISIPDFSGDFKIKHLGKGTYEFYSMAV 85
House mouse	1:MTWAPDNVRKWSALLLLAIIGTA-LTAATDPGFVAMISQKGLDFACQ	QVVELQKELQAISVPDFSGVFKIKHLGKGSYEFYSMAV 85
Dog	1:MARHPDNALRWATLLVLAILGTAAVVTTTNPGVVARITQKGLDYACQ	QGVAVLQKELEKIKIPTFSGSFKVKHLGKGHYSFYSMVI 86 XGVATLRKELEKITIPTFSGSFKIKYFGKGRYNFYSMVV 85
Pig Cattle	1:MARGADNTLRWATLVALAALGTA-VTAAANPGIVARITQKGLDYACQ 1:MARGPDTARRWATLVVLAALGTA-VTTT-NPGIVARITQKGLDYACQ	ZGVATLRKELEKITIPTFSGSFKIKYFGKGRYNFYSMVV 85 YZVLTLOVELEKITIPNESGNEKIKYLGKGOVSEESMVI 94
Human	1:MRENMARGPCNAPRWVSLMVLVAIGTA-VTAAVNPGVVVRISQKGLDYASQ	GTAALQKELKRIKIPDYSDSFKIKHLGKGHYSFYSMDI 89
Black rockfish	27 UNICI BEST DI UDOBOUELOTONS EL BRECKUBURY DI TERDOORDI NU	DISTRUCTOR SCHULDCHARDSSUCCENTERS 1.65
Olive flounder	77 VLGLPKSALDLVPGTGVKLSIGNAFLRMHGNWRVKYLRIIKDSGSFDLNVI 77 VKLGLPKSAVDLVPGTGIKLSISNAYISLHGNWRVKYLRIIKDSGSFDLNVI	ADDITITTTIAIKSDE-TGRPVVSSVNCAATVGSAKIKFH 165
Atlantic cod A	77:VKLGLPSSAIGLVPGTGFSLTITNAFLSLHGRWRVKYLRFIKDRGSFDLAI	KSLSITTSISLRSDN-MGLPAVAMASCTTTLGGVSVKLH 165
Atlantic cod B	77:VKLGLPSSAIGLVPGTGFSLTITNAFLSLHGRWRVKYLRFIKDRGSFDLAI	KSLSITTSISLRSDN-MGLPAVAMASCTTTLGGVSVKLH 165
Ayu	75: VNLGLPKSVLGLVPGTGVNLSIGNAFLNLHGNWRVRYLKFIKDSGSFDLGI	KGLTITTSIAIKSDE-TGRPSVSSINCVTSLGSATIKFH 163
Rainbow trout 1 Rainbow trout 2	77:VNLGLPYSALALVPDTGISLSITNAFISLHGNWKIRYLSFIKDSGSFDLEV 77:VNLGLPKSALVLMPGTGVRLAITNAFINLHGNWRVRYFRFIQDRGSFDLAVI	GLTVTDSITIKSDE-TGRFTVSSVNCVANVGSASIKFH 165
Common carp	77:VNLGLPKSALVLVPDTGVMLSIGNAYINLHGNWRVKYLRIIKDSGSFDLAV	SELTISTTVAVMSDD-TGHPTVSMTNCAATVGSVNVKFH 165
Channel catfish	79:LDLGLPKSAVGLVPGTGVSLSIGDAYINLHGNWRVKYLRIIKDSGSFDLSV	GLSISATISVKGDD-TGRPVVSSANCAASVGNVKIKFH 167
Norway rat	86: EGFHIPDPQIKLLPSDGLQLSITSASIKISGRWKYR-KNILKASGNFQLSI	QGVSIIADLILGNDP-SGRITITCSTCDSHINSVRIKVS 173
House mouse Dog	86: DGFHIFNPKIEMLPSDGLRVFIKDASIKINGKWMSR-KNFLKAGGNFELSI	2GVSISTDLILGSDS-SGHITTICSNCDSHIDSVHIKIS 173
Pig	87: RGFQLPSSQIKLVPNKGLDLSIRNANIKISGKWKAR-KNFIKTSGNFDLSVI 86: REFKLPTSQIRLSPDRGLDLSIKDASVKISGKWKAQ-KNFIKASGNFDLSVI	ERISVLASLELGYDPTSGHSTVSCSSCENHINEVHVETS 175
Cattle	85: QGFNLPNSQIRPLPDKGLDLSIRDASIKIRGKWKAR-KNFIKLGGNFDLSV	GISILAGLNLGYDPASGHSTVTCSSCSSGINTVRIHIS 173
Human	90:REFQLPSSQISMVPNVGLKFSISNANIKISGKWKAQ-KRFLKMSGNFDLSI	EGMSISADLKLGSNPTSGKPTITCSSCSSHINSVHVHIS 178
	** .** . ** **.*.*.*	* *
Black rockfish	166: GGA-SWLYNLFRKFVDKAIRNALOKOMCPLVANAVSD-LNPOLKTLNVLAK	DOVARTEY CMUSSION CONSCIENCE VGEEV MICKLOED 253
Olive flounder	166.GGA-SWLYNLFKNFIDKALRNALQKQICPLVADAVNE-LNPHLKTLNVLAK	DQYAEIEYSMVSSPTVSKSSIDLNLKGEFYNIGKHQEA 253
Atlantic cod A	166:GGA-SWLYNLFRRFIEKGLQSQLQKKLCPLVAESVSS-MNQFLKTVNVMAP	DRYAEIAYPMVSSPDISSSSIGLNLKGEFYNIGNHMEP 253
Atlantic cod B	166:GGA-SWLYNLFRRFIEKGLQSQLQKKLCPLVAESVSS-MNQFLKTVKVMAP	/DRYAEIAYPMVSSPDISSSSIGLNLKGEFYNIGNHMEP 253
Ayu Rainbow trout 1	164:GGA-SWLYNLFSSYIDKALRNSLQKKICPLVADAVTD-MNPHLKTLNVLAK 166:GGA-SWLYNLFSAYIDKALRSALQKQICPLVADTITD-MNPHLKTLNVLAK	/DKYAEIEYSMVESPAISKSCIDLGLKGEFYNVGAHQEP 251
Rainbow trout 2	166: GGA-SWLYNLFKSYIDKALRSALOKOICPLVADVITD-MNPHLKTENVLAK	DOYAEIEYSMVTSPTISNASIDESLKGEFINIGRHOEP 253
Common carp	166:GGA-SWLYNLFKSYIDKALRSALQKQICPLVADVITD-MNPHLKTFNVLAK 166:GGA-SWLYNLFSSFINKALRNALQKQICPLVADSIAD-INPHLKTLNVLAK	DQYAEIEYSMVGSPVISNTSIDLGLKGEFYNIGQHKEP 253
Channel catfish	168: GGA-SWLYNLFKHYIEKALRSELOKOICSLVAEAIEE-MNPHLKTLNVLAO	DKYAEIEYSMVESPLMSNSSIDLSLKGEFYNIGOHKEP 255
Norway rat House mouse	174:GSMLGWLIQLFHRKIETSLKKTIYKKICKIVRNSVSAKLQPYVKTLPVVAK 174:GSMLGWLIRLFHRKIETSLKNIIYKKICKIVRDSVSSKLQPYLKTLSVIRV	/DDITSIDYSLLAPPMTTDKFLEGQLRGEFFWRGHHGPF 263
Dog	174:GSMLGWLIQLFHKKIESSIRKTMNNKICQVVSSSVSSRLQSYLKTLPVTYK	DRIAGISYSLVAPPTATADNLDGHLKGEFFSLAHPRSP 265
Pig	175:GSSLKWLIQLFHRNIESALRKAMESKICKMLTNTVSSKLQPYFQTLPVTAK	DRMVGINYSLVAPPKATAENLDGLLKGEFFSLDHPSPP 264
Cattle	174:GSSLGWLIQLFRKRIESLLQKSMTRKICEVVTSTVSSKLQPYFQTLPVTTK	LDKVAGVDYSLVAPPRATANNLDWLLKGEFFSLAHRSPP 263
Human	179:KSKVGWLIQLFHKKIESALRNKMNSQVCEKVTNSVSSELQPYFQTLPVMTK	LDSVAGINYGLVAPPATTAETLDVOMKGEFYSENHHNPP 268
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Black rockfish	254 PFSPAAFSLPPOINNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPOA	* *
Black rockfish Olive flounder	254 PFS PAAFSL PPOTNNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPOA 254 PFS PTAFSL PPONDDMLYMGMSAYSVNSAAFVYNKAGALSLYITDDMIPOS	* *
Olive flounder Atlantic cod A	254 (PFSPAAFSLPPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPCA) 254 (PFSPTAFSLPPCNDDMLYMCMSAYSVNSAAFVYNKAGALSLYTTDDMIPC3) 254 (PFSPAPFLPNDRCSMLYIGLSAFTANSAFYVNKAGTLSLKTDDMIPC3)	* * * * * * * * * * * * * * * * * * *
Olive flounder Atlantic cod A Atlantic cod B	254 (PFSPARESLPPOINNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPOA 254 : PFSPTAFSLPPONDDMLYMGMSAYSVNSAAFVYNKAGALSLYITDDMIPOS 254 : PFSPAPFFLPNQEQSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 254 : PFSPAPFFLPNQEQSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS	* *** <u>SPIRLINTETGAFIPOVAKEPPGLMMKLLVKTAKNPVVT</u> 343 SPIRLINTEGFFIPEISKEPGLMMLLVQTVKTFVIT 343 SPIRLITINTGVLIPQIAKLEPGLMVLQLKMTNDFLSS 343 SPIRLITINTFOVLIPQIAKLEPGLMVLQLKMTNDFLSS 343
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1	254 (PESPARESLPPOINNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPOA 254 (PESPTAR SLPPONDMLYMGMSAYSVNSAAFVYNKAGALSLYITDDMIPOS 254 (PESPAPEFLENQEQSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 254 (PESPAPEFLENQEQSMLYIGLSAFTANSASFVYNKAGALSLYITDDMIPRS 252 (PESATPESLPPQISNMLYIGLSAFTNSAGFVYNKAGALSLYITDDMIPPS)	* *** SPIRLINTERFORFIPOVAKEPPGLMMKLLVKTAKNPVVT 343 SPIRLINTERFOFFIPEISKEPGLMMKLLVQTVKTFVIT 343 SPIRLITINTFOVLIPQIAKLEPGLMMVLQLKMTNDELSS 343 SPIRLITINTFOVLIPQIAKEPGLMMKLLVKTVQEPIIS 341 SPIRLINTERFORFIPQIAKEPGLMMKLLVKTVQEPIIF 341
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2	254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA 254 (PFSPTAFSLEPONDMLYMGMSAYSVNSAAFVYNKAGALSLYTTDDMIPQS 254 (PFSPAFFLENDECSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 252 (PFSPAFFLENDECSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 252 (PFSPAFFSLEPOLSMLYIGLSAFTANSASFVYNKAGALSLYTDDMIPPS 254 (PFSPTFSLEPOUNMLYIGMSAFTINSAGFVYNNAGALSLYTDDMIPPS)	* *** <u>PIRLINTETEGAEIPCVAKEPGLMMKLLUKTAKNPVT</u> 343 SPIRLINTEGTFIPEISKRPGLMMKLLUKTVKTPVT 343 SPIRLITINTFGVLIPQLAKLEPGLMMVLQLKMTHDPLSS 343 SPIRLITINTFGAIFIQLAKLEPGLMMVLQLKMTHDPLSS 343 SPIRLITINTFGAFIPQLAKEPFIMMKLLUKTVKEPTIT 341 SPIRLINTETFGAFIPELAKEPFSMMKLLUKTVKEPTIS 343
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp	254 (PESPARESLPPOINNMLYISVSAFTINSAAFYYNTAGALSLYITDDMIPQA 254 (PESPARESLPFQNDMLYMGMSAYSVNSAAFYYNKAGLSLYITDDMIPQS 254 (PESPAPEFLENQEQSMLYIGLSAFTANSASFYYNKAGTLSLKITDDMVPRS 255 (PESATPFSLPPQISNLYIGLSAFTANSASFYYNKAGLSLYUTDDMIPRS 254 (PESATPFSLPQUSNLYIGLSAFTANSAGFYYNKAGALSLYUTDDMIPPS 254 (PESPTPFSLPQUNNLYIGMSAFTINSAGFYYNNAGALSLYUTDDMIPPS 254 (PESPTPFSLPQDNNLYIGVSAFTINSAGFYYNNAGALSLYUTDDMIPPS 254 (PESPTPFSLPQDNNLYIGVSAFTINSAGFYYNNAGALSLYUTDDMIPS)	* *** SPIRLINTETFORFIPOVAKRPPGLMMKLLVKTAKNPVVT 343 SPIRLINTETFOTFIPEISKRPGLMMKLLVQTVKTPVIT 343 SPIRLINTETFOVIIPQIAKLPGLMMVLQLKMTNDPLSS 343 SPIRLINTETFOVIIPQIAKRPGLMMKLLVKTVQEPIIS 343 SPIRLINTETFOAFIPQIAKRPGLMMKLLVKTVKEPTIS 343 SPIRLINTETFOAFIPEIAKRPGMMMKLLVKTVKEPTIS 343 SPIRLINTETFOAFIPEIAKRPGMMMKLLVETVKEPTIS 343
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish	254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA, 254 (PFSPTAFSLEPONDMLYMGMSAYSVNSAAFVYNKAGALSLYTTDDMIPQS, 254 (PFSPAFFLENDECSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS, 252 (PFSPAFFLENDECSMLYIGLSAFTANSASFVYNKAGALSLYTDDMIPS), 252 (PFSPAFFSLEPOLSMLYIGLSAFTANSASFVYNKAGALSLYTDDMIPS), 254 (PFSPTFFSLEPONNMLYIGMSAFTNSAGFVYNNAGALSLYTDDMIPS), 254 (PFSPTFFSLEPONNMLYIGVSSFTNSAGFVYNNAGALSLYTDDMIPS), 254 (PFSPTFFSLEPONNMLYIGVSSFTNSAGFVYNNAGALSLYTDDMIPS), 254 (PFSPTFFSLEPONNMLYIGVSSFTNSAGFVYNNAGALSLYTDDMIPS),	* *** <u>PIRLINTETFGAFIPQVAKRFPGIMMKLLVKAKNPVVT</u> 343 SPIRLINTEGTFIPEISKRFPGIMMKLLVGTVKTPVT 343 SPIRLITNTFGVIIPQIAKLFPGIMMVLQLKMTNDPLSS 343 SPIRLITNTFGAFIPQIAKLFPGIMMKLLVKTVCEFIT 341 SPIRLINTETGAFIPEIAKRFPSMMKLLVKTVCEFIT 343 SPIRLINTETGAFIPEIAKRFPSMMKLLVKTVCEFIT 343 SPIRLINTETGAFIPQIEKMYPGIMMKLLVKTVCEFIT 343 SPIRLINTETGAFIPQIEKMYPGIMMLLVKAECEFIVS 345
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse	254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA, 254 (PFSPAAFSLEPONDMLYMGMSAYSVNSAAFVYNKAGALSLYTTDDMIPQS, 254 (PFSPAPFLENOECSMLYIGLSAFTANSASFVYNKAGTLSLKITDDMVPRS; 252 (PFSPAPFLENOECSMLYIGLSAFTANSASFVYNKAGALSLYTDDMIPS; 252 (PFSPAFFSLEPQUSNLYIGLSAFTANSASFVYNKAGALSLYTDDMIPS; 254 (PFSPTFSLEPQUNNLYIGMSAFTNSAGFVYNNAGALSLYTDDMIPS; 254 (PFSPTFSLEPQUNNLYIGVSSFTNSAGFVYNNAGALSLYTDDMIPS; 254 (PFSPTFSLEPQDNNLYIGVSSFTNSAGFVYNNAGALSLYTDDMIPS; 256 (PFSPTFSLEPQDNNLYIGUSSFTINSAGFVYNNAGALSLYTDDMIPS; 256 (PFSPTFSLEPQDSNMYIGLSAFTLNSAGFVYNNAGALSLYTDDMIPS; 264 (PAUPPUNRUPDNSMYWCMGISDYFFNTAFFAYQESETLKTILRQLLAKD);	* *** SPIRLINTETGAFIPOVAKRFPGLMMKLLVKTAKNFVVT 343 SPIRLINTEGAFIPOVAKRFPGLMMKLLVGVVKTPVT 343 SPIRLITINTEGVLIPOJAKLFPGLMMVLOLKMTNDELSS 343 SPIRLITINTEGVLIPOJAKRFPGLMMKLLVKTVOEPIIT 341 SPIRLINTETGAFIPOJAKRFPGLMMKLLVKTVEFTIF 343 SPIRLINTETGAFIPEIAKRFPGMMKLLVKTVEFTIF 343 SPIRLINTETGAFIPEIAKRFPGLMMKLLVKTVEFTIF 343 SPIRLINTETGAFIPOJEKMYPGLMMKLLVKTVEFTIF 343 SPIRLINTETGAFIPOJEKMYPGLMMKLLVKAFENVS 345 RININTEFGFIPIOJEKMYPGLMMKLLVKAFENVS 345 RININTEFFFIPIOJEKMYPGLMMKLLVKAFENVS 353 SRPOLNTDFLKTFLPEVAKKFPSMGLOLLISAPUFAHLN 353 SRPOLNTDFLKTFLPEVAKKFPSMGLOLLISAPUFAHLN 353
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog	254 (PESPARESLPPOINNMLYISVSAFTINSAAFYYNTAGALSLYITDDMIPOA 254 (PESPARESLPFONDMLYMGMSAYSVNSAAFYYNKAGALSLYITDDMIPOS 254 (PESPAPEFLENOCOSMLYIGLSAFTANSASFYYNKAGTLSLKITDDWVPRS 254 (PESPAPEFLENOCOSMLYIGLSAFTANSASFYYNKAGALSLYITDDMIPS 254 (PESPAPEFLENOCOSMLYIGLSAFTANSASFYYNKAGALSLYITDDMIPS 254 (PESPIPESLPFQVNNKLYIGMSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESLPFQVNNKLYIGMSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESLPFQDTNKLYIGMSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESLPFQDTNKLYIGMSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESLSQDTDKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESPAQDTNKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESPAQDTNKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESPAQDTNKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPENSLAGDTNKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESPAQDTNKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PESPIPESPADALPODTNKLYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PENTRUPYNNILYIGUSAFTINSAGFYYNNAGALSLYITDDMIPS 254 (PENTRUPYNNILYIGUSAFTINSAGFYNNAGALSLYITDDMIPS 254 (PENTRUPYNNALYIGUSAFTINSAGFYNNAGALSLYITDDMIPS 255 (PESPIPESLAFUNNAYKYGUSISYFNAFTNAGALSLYITDDMIPS 264 (PINPPNALAPPONALYNCMGISDYFNAFTNAGALSLAMITTDOMIPS 265 (PESPIPEAALPPONALNYNCMGISDYFNAGULYQQSGILMMITIGGLLSNM	**** SPIRLINTETFGAFIPOVAKRPPGLMMKLLVKTAKNPVVT 343 SPIRLINTETGTFIPEISKRPGLMMKLLVQTVKTPVIT 343 SPIRLINTETGVIIPQIAKLPFGLMMVLQLKMTNDFLSS 343 SPIRLINTETGAFIPQIAKRPGLMMKLLVKTVCEPTIF 341 SPIRLINTETGAFIPQIAKRPGMMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPEISKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPQIAKRPGMMMLLVKTVCEPTIF 345 SPIRLINTETGAFIPUT 345 SPIRLINTETG
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig	254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA 254 (PFSPAAFSLEPONDMLYMGMSAYSVNSAAFVYNKAGALSLYTTDDMIPQS 254 (PFSPAFFLENDEGSMLYTGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 252 (PFSPAFFLENDEGSMLYTGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 252 (PFSPAFFSLEPOLSMLYTGLSAFTANSASFVYNKAGALSLYTDDMIPS 254 (PFSPTFSLEPOUNMLYTGMSAFTNSAGFVYNNAGALSLYTDDMIPSS 254 (PFSPTFSLEPOUNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPSS 254 (PFSPTFSLEPODNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPSS 256 (PFSPTFSLEPODNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPSS 256 (PFSPTFSLEPODSNMYTGLSAFTINSAGFVYNNAGLSLYTDDMIPSS 266 (PFTPEAMALEPODSNMYTGUSSFTNSAGFVYNNAGLSLYTDDMIPSS) 264 (PAUPPVMRILPNNNYMCMGISDYFFNTAFFAYGESTLKMITAGOLSNM 266 (PTTPEAMALEPODDISMVTGUSSFFTNSGLVYQEAGALMMITTDDMIPSS) 265 (PFSPEALEPODDISMUSSFFNTAGLVYQEAGALMMITTDDMIPSS)	**** **** **** **** **** **** **** **** ****
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog	254 (PFSPAAFSLFPOINNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPQA 254 (PFSPAAFSLFPOINNMLYISVSAFTINSAAFVYNKAGALSLYITDDMIPQS 254 (PFSPAFFLNG©SNLYIGLSAFTANSASFVYNKAGALSLXITDDMVPRS 254 (PFSPAFFLNG©SNLYIGLSAFTANSASFVYNKAGALSLXITDDMVPRS 254 (PFSPAFFSLFQUSNLYIGLSAFTANSASFVYNKAGALSLVIDDMVPRS 254 (PFSPTFSLPQUSNLYIGLSAFTANSASFVYNKAGALSLVIDDMVPRS 254 (PFSPTFSLPQUSNLYIGLSAFTANSASFVYNKAGALSLVIDDMVPRS 254 (PFSPTFSLPQUSNLYIGVSYTNSAGFYYNKAGALSLVIDDMVPRS 254 (PFSPTFSLPQUSNLYIGVSYTNSAGFYYNKAGALSLVIDDMIPS) 254 (PFSPTFSLPQUSNLYIGVSYTNSAGFYYNKAGALSLVIDDMIPS) 254 (PFSPTFSLPQUSNLYIGVSYTNSAGFYYNKAGALSLVIDDMIPS) 254 (PFSPTFSLPQUSNLYIGVSYTNSAGFYYNKAGALSLVIDDMIPS) 254 (PFSPTFSLPQUSNLYIGVSYTNSAGFYYNKAGALSLVIDDMIPS) 264 (PUNNNYKYGNISYYFNTSVLAYQQSSTLKMTLGGLLSNM 264 (PTPPAMALPPUNNKYKGISSYFFNTAGLYYQKASVLMLTINSMIPKX 265 (PFAPPALALPADHIRWYYLGISSYFFNTAGLYYQKASVLMLTINSMIPKX 264 (PFAPPALALPADHIRWYYLGISSYFFNTAGLYYQKASVLMLTINSMIPKX	**** SPIRLINTETFGAFIPOVAKRPPGLMMKLLVKTAKNPVVT 343 SPIRLINTETGTFIPEISKRPGLMMKLLVGTVKFVIT 343 SPIRLINTETGTFIPEISKRPGLMMKLLVGTVKFVIT 341 SPIRLINTETGAFIPOLAKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPEISKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 343 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGFFIPOLAKRPGMMLLVETVCEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVKTVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVKTVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVETVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVETVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLUFTVASSPFFILT 355 SIFFLITTEFGFILIPOVAKRPFIFIMOLOFIFIAMSSFFFILT 355 SIFFLITTEFFFILTFV SFREMPFIFI
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle	254 (PFSPAAFSLEPOINNMLYISVSAFTINSAAFYYNTAGALSLYITDDMIPQA, 254 (PFSPAAFSLEPONDMLYMGMSAYSVNSAAFYYNKAGALSLYITDDMIPQS, 254 (PFSPAPFLENOECSMLYIGLSAFTANSASFYYNKAGTLSLKITDDMVPRS; 254 (PFSPAPFLENOECSMLYIGLSAFTANSASFYYNKAGALSLYITDDMIPS; 254 (PFSPAFFSLEPQUNNLYIGLSAFTANSASFYVNKAGALSLYTDDMIPS; 254 (PFSPTPSLEPQUNNLYIGNSAFTINSAGFYVNNAGALSLYITDDMIPS; 254 (PFSPTPFSLEPQUNNLYICVSSFTNSAGFYVNNAGALSLYITDDMIPS; 256 (PFSPTPSLEPQDNLYICVSSFTNSAGFYVNNAGALSLYITDDMIPS; 256 (PFSPTPSLEPQDNNLYICVSSFTNSAGFYVNNAGALSLYITDDMIPS; 266 (PFSPTPSLEPQDSNMYIGLSAFTLNSAGFYVNNAGLSLYITDDMIPS; 266 (PFNPAMALEPDDHIMWYLCGISDYFNNAFFAVQESETLKITLRQLLAKD) 266 (PFNPAMALEPDHIMWYLCGISSYFTNAGLYVQEAGALMMITTDDMIPK; 265 (PFSPAMALEPDHIMWYLCGISSYFFNNAGLYVQEAGALMMITTDDMIPK; 266 (PFNPAMALEPDHIMWYLGISSYFFNNAGLYVQEAGALMMITTDDMIPK; 266 (PFNPAMALEPDHIMWYLGISSYFFNNAGLYVQEAGALMULTINNMIPK; 266 (PFAPAMALEPDHIMWYLGISSYFFNNAGLYVQEAGALMULTINNMIPK; 266 (PFAPAMALEPDHIMWYLGISSYFFNNAGLYVQEAGALMULTINDMIPK; 266 (PFAPAMALEPDHIMWYLGISSYFFNNAGLYVQEAGALMULTINDMIPK; 266 (PFAPAMALEPDHICLSYFFNNAGLYVQEAGALMULTINDMIPK; 267 (PFAPAMALEPDHIMWYLGISSYFFNNAGLYVQEAGALMULTINDMIPK; 268 (PFAPAPALALEPSHICLSSYFFNNAGLYVQEAGALMULTINDMIPK; 269 (PFAPAMALEPDHICLSSYFFNNAGLYVQEAGALMULTINDMIPK; 264 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 264 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 265 (PFAPAMALEPAHICMWYLGISSYFFNNAGLYVQEAGALMULTINDMIPK; 266 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 266 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 266 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 266 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 267 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 268 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 269 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 260 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 261 (PFAPPAMALEPAHICMULGISSYFFNNAGLYVQEAGALMULTINDMIPK; 261 (PFAPPAMALEPAHICMULGISSYFFNAGLYVQEAGALMULTINDMIPK; 261 (PFAPPAMALEPAHICMULGISYFFNAGLYVQEAGALMULTINDMIPK; 261 (PFAPPAMALEPAHICMULGISYFFNAGLYVQEAGALMULTINDMIPK; 261 (P	**** SPIRLINTETFGAFIPOVAKRPPGLMMKLLVKTAKNPVVT 343 SPIRLINTETGTFIPEISKRPGLMMKLLVGTVKFVIT 343 SPIRLINTETGTFIPEISKRPGLMMKLLVGTVKFVIT 341 SPIRLINTETGAFIPOLAKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPEISKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMKLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEPTIF 343 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 343 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGAFIPOLAKRPGLMMLLVKTVCEFTIF 345 SPIRLINTETGFFIPOLAKRPGMMLLVETVCEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVKTVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVKTVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVETVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLLVETVEFTV 345 SPIRLINTETGFFIFIPOLAKRPGMUKLUFTVASSPFFILT 355 SIFFLITTEFGFILIPOVAKRPFIFIMOLOFIFIAMSSFFFILT 355 SIFFLITTEFFFILTFV SFREMPFIFI
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human	254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA 254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNKAGALSLYTTDDMIPQS 254 (PFSPAFFLENCECSHLYTGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 254 (PFSPAFFLENCECSHLYTGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 252 (PFSPAFFSLEPOISNMLYTGLSAFTANSASFVYNKAGALSLYTDDMIPPS 254 (PFSPTFSLEPOINNLYTGMSAFTINSAGFVYNKAGALSLYTDDMIPPS 254 (PFSPTFSLEPOINNLYTGVSFTINSAGFVYNKAGALSLYTDDMIPPS 254 (PFSPTFFSLEPOINNLYTGVSFTINSAGFVYNKAGALSLYTDDMIPS 256 (PFSPTFSLEPOINNLYTGVSFTINSAGFVYNKAGLSLYTDDMIPS 256 (PFSPTFSLEPOINNLYTGUSFTINSAGFVYNKAGLSLYTDDMIPS 266 (PFTPPANALEPOINNLYTGUSFTINSAGFVYNKAGLSLYTDDMIPS 266 (PTTPPANALEPOINNLYTGUSFTINSAGFVYNKAGLSLYTDDMIPS 266 (PTTPPANALEPOINNLYTGUSFTINSAGFVYNKAGLSLYTDDMIPS 266 (PTTPPANALEPOINNLYTGUSFTINSAGFVYNKAGLSLYTDDMIPS 266 (PTTPPANALEPOINNLYTGUSFTINTAGLYVQEAGALMITTDDMIPKS 266 (PTTPPANALEPOINTHYTGUSTSFTINSGUSVCEAGUNLTTNMIPKK 266 (PTTPPANALEPOINTHYTGUSSFTINTAGLYVCKAGALNLTTDMIPKS 266 (PTTPPANALEPOINTHYTGUSSFTINTAGUNVCKAGALNLTTDDMIPKS 266 (PTTPPANALEPOINTHYTGUSSFTINTAGUNVCKAGALNLTTDDMIPKS 266 (PTTPPANALEPOINTHYTGUSSFTINTAGUNVCKAGALNLTTDDMIPKS) 267 (PTPPANALEPOINTHYTGUSSFTINTAGUNVCKAGALNLTTDDMIPKS) 268 (PTPPANALEPOINTHYTGUSSFTINTAGUNVCKAGALNLTTDDMIPKS) 269 (PTAPPANALEPOINTHYTGUSSFTINTAGUNVCKAGALNLTTDDMIPKS) 269 (PTAPPANALEPOINTHYTGUSSTFTNTAGUNVCKAGALNLTLRDDMIPKS)	***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***     ***
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat Bog Dog Cattle Human Black rockfish	254 PFSPAAFSLFPONNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA 254 PFSPAAFSLFPONDMLYMMSAYSVNSAAFVYNKAGALSLYTTDDMIPQS 254 PFSPAFFLNGCSMLYTGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 254 PFSPAFFLNCGCSMLYTGLSAFTANSASFVYNKAGTLSLKITDDMVPRS 254 PFSPAFFSLPONNMLYTGNSFTINSAGFVYNKAGALSLVTDDMIPS 254 PFSPTFSLPONNMLYTGMSAFTNSAGFVYNKAGALSLVTDDMIPS 254 PFSPTFSLPONNMLYTGMSAFTNSAGFVYNKAGALSLVTDDMIPS 255 PFSPTFSLPONNMLYTGMSAFTNSAGFVYNKAGALSLVTDDMIPS 256 PFSPTFSLPONNMLYTGMSAFTNSAGFVYNKAGALSLVTDDMIPS 256 PFSPTFSLPONNMLYTGMSAFTNSAGFVYNKAGALSLVTDDMIPS 266 PFTFSLPONNMLYTGNSFTINSAGFVYNKAGALSLVTDDMIPS 266 PFTFSLPONNMLYTGNSFTINSAGFVYNKAGULSLVTDDMIPS 266 PFTFSLPONNMLYTGNSFTINSAGFVYNKAGULSLVTDDMIPS 266 PFTPSLPONNMLYTGNSFTINSAGFVYNKAGULSLVTDMIPS 266 PFAPPLALSTUNGTUSSTTNSAGFVYNKAGULSLVTDMIPS 266 PFAPPLALSTUNGTUSSTTNSAGFVYNKAGULSLVTDMIPS 266 PFAPPLALSTUNGTUSSTTNSAGFVYNKAGULSLVTDMIPS 269 PFAPPLALSTUNGTUSSTTNSAGFVYNKAGULSLVTDMIPS 269 PFAPPLALSTUNGTUSSTTNSAGFVYNKAGULSLVTDMIPS 269 PFAPPLALSTUNGTUSSTTNSAGFVYNKAGULSLUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGFVYNKAGULSLTINNMIPS 269 PFAPPLAFSTUNGTUSSTTNSAGFVYNKAGULSLUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGTVYKAGTUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGTVYKAGTUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGTVYKAGTUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGTVYKAGTUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGTVYKAGTUNGTUSST 269 PFAPPLAFSTUNGTUSSTTNSAGTVYKAGTUNGTUSST 269 PFAPPLAFSTUNGTUSST 269 PFAPLAFSTUNGTUSST 269 PFAPLAFST 269 PF	****  SPIRLINTETFGAFIPOVAKRFPGLMMKLLVKTKKNPVVT 343 SPIRLINTETFGTFIPEISKRFPGLMMKLLVGTVKTVT 343 SPIRLINTETFGTFIPEISKRFPGLMMKLLVGTVKTVDES 343 SPIRLINTETFGAFIPOLAKRFPGLMMKLLVKTVCEPTIF 341 SPIRLINTETFGAFIPOLAKRFPGLMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGLMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGLMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGLMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGLMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGMMKLLVKTVCEPTIF 343 SPIRLINTETFGAFIPOLAKRFPGMMKLLVKTVCEPTIF 343 SPIRLINTEFGAFIPOLAKRFPGMMKLLVETVCEFTV 345 SRFQLTTFFFGAFIPOLAKRFPMKTGLLVSTVEFTV 353 SRFQLTTFFFGILIPOVAKRFPMEMGPLIMASFPHLI 354 SKFRLTTFFFGILIPOVAKRFPMKKDQLFFVASASTPFHLS 358 SKFRLTTFFFGILIPOVAKRFPMKKDQLFVASASTPFHLS 358 SKFRLTTFFFGILFFQVAKRFPMKKDQLFVASASTPFHLS 358 SKFRLTTFFFGILFFQVAKRFPMKKDQLFVASASTPFHLS 358 SKFRLTTFFFGILFFQVAKRFPMKKDQLFVASASTPFHLS 358 SKFRFFFFGILFFQVAKRFPMKKDQLFVASASTPFHLS 358 SKFRFFFFFGILFFQVAKRFPMKF
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Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human Black rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1	<ul> <li>254 (PFSPAAFSLEPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA,</li> <li>254 (PFSPTAFSLEPONDMLYMGMSAYSVNSAAFVYNKAGALSLYTTDDMIPQS,</li> <li>254 (PFSPAFFLENDEGSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS;</li> <li>254 (PFSPAFFLENDEGSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS;</li> <li>254 (PFSPAFFSLEPONNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPS;</li> <li>254 (PFSPTFSLEPONNMLYTGVSFTINSAGFVYNKAGALSLYTDDMIPS;</li> <li>254 (PFSPTFSLEPONNMLYTGVSFTINSAGFVYNKAGLSLYTDDMIPS;</li> <li>256 (PFSPTFSLEPONNMLYTGUSSFTINSAGFVYNKAGLSLYTDDMIPS;</li> <li>266 (PFTPPANALEPONNMLYTGUSSFTINSAGFVYNKAGLSLYTDDMIPS;</li> <li>264 (PAPPVNILLYNNYMCMGISDYFFNTAEFAYDSETLKTILDDLLAKD</li> <li>264 (PAPPALALEPOHDHWYLGISDYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>265 (PFTPPANALEPOHDHWYLGISSYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>266 (PFTPPANALEPOHDHWYLGISSYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>267 (PFAPALALFSDHDRWYLGISSYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>268 (PFAPPALALFSDHDRWYLGISSYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>269 (PFAPPALALFSDHDRWYLGISSYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>264 (PAPPVNETPAHDRWYLGISSYFFNTAGLVYQEAGALMTTDDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGISSYFFNTAGLVYQEAGALMTTTDDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGISSYFFNTAGLVYQEAGALMTTTDDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGISSYFFNTAGLVYQKAGALMLTRDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGISSYFFNTAGLVYQKAGALMLTRDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGISSYFFNTAGLVYQKAGALMLTRDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGISSYFFNTAGLVYQKAGALMLTRDMIPK;</li> <li>264 (PAPPVAEFAHDRWYLGNTSLEPLEVLM,DFSVSARVVYCSKLI)</li> <li>274 (PEPNKVTVQAKSSLTAYATLANTKLAPLEVLML,DFSVSARVVYCSKLI)</li> <li>274 (PEPNNVTVQAKSSLTAYATLANTKLAPLEVLML,DFSVSARVVYCSKLI)</li> <li>274 (PEPNNVTVQASSLTAYATLANTKLAPLEVLML,DFSVSARVYVCSKLI)</li> <li>274 (PEPNNVTVQASSLTAYATLANTKLAPLEVLML,DFSVSARVYVCSKLI)</li> <li>274 (PEPNNVTVQASSLTAYATLANTKLAPLEVLML,DFSVSARVYVCSKLI)</li> <li>274 (PENNVTVQASSLTAYATLANTKLAPLEVLML,DFSVSARVYVCSKLI)</li> <li>274 (PENNVTVQASSUTAYATLOPNTLSPLEVLMLCASVSARVYVCSKLI)</li> <li>274 (PENNVTVQASSUTAYATLOPNTLSPLEVLML)DSVSARVYVCSKLI)</li> </ul>	**** **** **** **** **** **** **** **** ****
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human Black rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2	254 PESPARSLEPOINNMLYISVSAFTINSAAFYYNTAGALSLYTTDDMIPQA 254 PESPARFELPOINNMLYISVSAFTINSAAFYYNKAGALSLYTTDDMIPQS 254 PESPARFELPOICSMLYIGLSAFTANSASFYYNKAGTLSLKITDDWPRS 254 PESPARFELPOICSMLYIGLSAFTANSASFYYNKAGTLSLKITDDWPRS 254 PESPAFFELPOISMLYIGLSAFTANSASFYYNKAGALSLYTDDMIPS 254 PESPAFFELPOISMLYIGLSAFTINSAGFYYNKAGALSLYTDDMIPS 254 PESPTFSLPQUNNLYIGVSSFTNSAGFYYNKAGALSLYTDDMIPS 254 PESPTFSLPQUNNLYIGVSSFTNSAGFYYNKAGALSLYTDDMIPS 256 PESPTFSLPQDNNLYIGVSSFTNSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPQDSNMLYIGLSAFTINSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPQDSNMLYIGLSAFTINSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPQDSNMLYIGUSSFTNSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPQDSNMLYIGUSSFTNSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPDDSNMLYIGUSSFTNSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPDDSNMLYIGUSSFTNSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPDDSNMLYIGUSSFTNSAGFYYNKAGLSLYTDDMIPS 266 PESPTFSLPDDSNMLYIGUSSTFNNAGLYYQKAGUSLITINDMIPS 266 PESPTFSLPDDDNAYLGUSSTFNNAGLYYQKAGULNLTINDMIPS 266 PESPTPSLDDNAYLGUSSTFNNAGLYQKAGVLNLTINDMIPS 267 PESPARALEPENDENYYLGUSSTFNNAGLYQKAGVLNLTINDMIPS 268 PERPENALEPENDENYYLGUSSTFNNAGLYQKAGVLNLTINDMIPS 269 PERPENALEPENDENYLGUSSTFNNAGLYQKAGVLNLTINDMIPS 344 PEENKUTVQATSTUTAVAIQENTILSPLFLILNETSVSARVEVSGML 344 PEENKUTVQAKSSLTAVAILANTKLAPLEVLNLDESVSARVEVSGML 344 PENNKUTVDAKSSLTAVAILANTKLAPLEVLNLDESVSARVEVSGML 344 PENNKUTVQARSSUTAVAILANTKLAPLEVLNLDESVSARVEVSGML 344 PENNKUTVQARSSUTAVAILANTKLAPLEVLNLESVSARVEVSGML 344 PENNKUTVQARSSUTAVAILANTKLAPLEVLNLESVSARVEVSGML	**************************************
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human Black rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1	254 PFSPARFSLFPONNMLYTSVSAFTINSAAFVYNTAGALSLYTTDMTPOA 254 PFSPARFELPONNMLYTSVSAFTINSAAFVYNKAGALSLYTTDMTPOA 254 PFSPAFFLNQCSMLYTGLSAFTANSASFVYNKAGTLSLKTDMVPRS 254 PFSPAFFLNQCSMLYTGLSAFTANSASFVYNKAGTLSLKTDMVPRS 254 PFSPAFFSLPQCISMLYTGLSAFTANSASFVYNKAGALSLVTDMVPRS 254 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGALSLVTDMIFPS 254 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGALSLVTDMIFPS 256 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGALSLVTDMIFPS 266 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGALSLVTDMIFPS 266 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGLSLVTDMIFPS 266 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGLSLVTDMIFPS 266 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGLSLVTDMIFPS 266 PFSPTFSLPQONNMLYTGVSAFTINSAGFVYNNAGULSLVTDMIFPS 266 PFSPTFSLPQDSNMYTGUSSYFNTAEFAVGESTLKMTTAGGLSLNTDMIPS 266 PFSPAALDEDHDRWYLGISVFFNTAEFAVGESTLKMTTAGGLSLSW 265 PFSPAALDEDHDRWYLGISVFFNTAGLVYGKAGULNLTINDMIFKS 264 PARFPAALAFSDHDRWYLGISSYFFNTAGLVYGKAGULNLTINDMIFKS 265 PFSPTFSLPGDDRWYLGISSYFFNTAGLVYGKAGULNLTINDMIFKS 264 PARFPAALAFSDHDRWYLGISSYFFNTAGLYGKAGULNLTINDMIFKS 264 PARFPAALAFSDHDRWYLGISSYFFNTAGLYGKAGULNLTINDMIFKS 264 PARFPAALAFSDHDRWYLGISSYFFNTAGLYGKAGULNLTINDMIFKS 264 PARFPAALAFSDHDRWYLGISSYFFNTAGLYGKAGULNLTINDMIFKS 265 PFSFFNTAGFVTTAVALOPNTLSELFULNLETSVSARVFYSGKHL 344 PENKTYVDASSLTAVALLANKLAFLEVLNLDFSVSARVVYGKHL 344 PENKTYDASSLTAVALLANKLAFLEVLNLDFSVSARVVYGKHL 344 PENNTVQASGSVTAVATGPNTLSELFULNLESSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLESSVSARVTYGKKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLESSVSARVTYGKKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLESSVSARVTYGKKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLESSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLENSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLENSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLENSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLENSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLENSVSARVTYGKL 344 PENNTVQASSTVTAVATGPNTLSELFULNLENSVSARVTYGKL	**************************************
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human Black rockfish Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat	254 PFSPAAFSLFPOINNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPQA 254 PFSPTAFSLFONDNULYIGVSAFTINSAAFVYNKAGALSLYITDDMIPQA 254 PFSPAFFLONGOSNLYIGLSAFTANSASFYVNKAGALSLYITDDMIPGS 254 PFSPAFFLONGOSNLYIGLSAFTANSASFYVNKAGALSLYITDDMIPGS 254 PFSPAFFSLPOINNLYIGLSAFTANSASFYVNKAGALSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGLSAFTANSASFYVNKAGALSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNKAGALSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNKAGALSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNKAGALSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNNKAGALSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNNKAGLSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNNKAGLSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNNKAGLSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNNKAGLSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNSAGFYVNNKAGLSLVITDDMIPGS 254 PFSPTFSLPOINNLYIGUSSFTNAGLYQQAGALMMITGDLIAKD 264 PTHPPMALPFOINNYKOMISDYFFNTAGLYQQAGALMMITGDNIPKS 265 PFAPPALAFSDHIRMYYGJSSYFFNTAGLYQQAGALMITGNDIPKS 269 PFAPPALAFSDHIRMYYGJSSYFFNTAGLYQQAGALMUTLRDMIPKS 269 PFAPPALAFSDHIRMYYGJSSYFFNTAGLYQQAGALMUTLRDMIPKS 269 PFAPPALAFSSLTAYAILANTKLAPLEVLNLETSVSARVFVSGKRLJ 244 PCPNNTLGASSTVTAYAIOPNTLSPLFILMLETSVSARVFVSGKRLJ 244 PCPNNTLAGSSTVTAYAIOPNTLSPLFILMLETSVSARVFVSGKRLJ 244 PSVSARVVVGKKLJ 244 PSVSARVVVGKKLJANTALANTKLAPLEVLNLBSVSARVVVGKKLJ 245 PFENNVTQASSSTVTAYAIOPNTLSPLFILMLESVSARVVVGKKLJ 246 PFENNVTQASSSTVTAYAIOPNTLSPLFULMLESVSARLVVGKKLJ 246 PFENNVTQASSTVTAYAIOPNTLSPLFULMLBSVSARLVVGKKLJ 246 PFENNVTQASSTVTAYAIOPNTLSPLFULMLBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFULMLBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVINBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVINBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVINBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVINBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVINBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVINBSVSARLVVGKKLJ 246 PENNVTQASSTVTAYAIOPNTLSPLFUVIN	****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      *****      *****      *****      ******
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human Black rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse	254 PFSPARFSLFPONNMLYTSVSAFTINSAAFVYNTAGALSLYTTDMTPOA 254 PFSPARFSLFPONDMLYMMSAYSVNSAAFVYNKAGALSLYTTDMTPOA 254 PFSPAFFLNQCSMLYTGLSAFTANSAFVYNKAGTLSLKTDDMVPRS 254 PFSPAFFLNQCSMLYTGLSAFTANSASFVYNKAGTLSLKTDDMVPRS 254 PFSPAFFSLPQUSMLYTGLSAFTANSASFVYNKAGALSLYTDDMTPS 254 PFSPTFSLPQUSMLYTGLSAFTNSAGFVYNKAGALSLYTDDMTPS 254 PFSPTFSLPQUNMLYTGVSFTNSAGFVYNNAGALSLYTDDMTPS 254 PFSPTFSLPQUNMLYTGVSFTNSAGFVYNNAGALSLYTDDMTPS 256 PFSPTFSLPQUSMLYTGLSAFTINSAGFVYNNAGALSLYTDDMTPS 266 PFSPTFSLPQUSMLYTGLSAFTINSAGFVYNNAGALSLYTDDMTPS 266 PFTFSLPQDSNMYTGUSAFTINSAGFVYNNAGLSLYTDDMTPS 266 PFTFPLADDSNMYTGLSAFTINSAGFVYNNAGLSLYTDDMTPS 266 PFTFPLADLADDSNMYTGLSAFTINSAGFVYNNAGLSLYTDDMTPS 266 PFTFPLADLADDSNMYTGUSAFTINSAGFVYNNAGLSLYTDDMTPS 266 PFTFPLADLADDSNMYTGUSAFTINSAGFVYNNAGLSLYTTDMTPS 266 PFTFPLADLADDSNMYTGUSAFTINSAGFVYNNAGLSLYTTDMTPS 266 PFTFPLADLADDSNMYTGUSAFTINSAGFVYNNAGLSLYTTDMTPS 266 PFTFPLADLADDSNMYTGUSAFTNAGLYQGAGULMTTTDDMTPS 267 PFTPLADLADDSNMYTGUSAFTNAGLYQGAGULMTTTDDMTPS 268 PFNFPLALADFSDHDRMYLGUSAFTNAGLYQGAGULMTTTDMTPKS 344 PENKTVUAGTSVTAVALOPNTLSPLFVLNLETSVSARVFVSGHL 344 PENKTVUASSLTAVALLANTKLAPLEVLNLDFSVSARVNVGHKL 344 PENNTVQASSLTAVALLANTKLAPLEVLNLDFSVSARVNVGHKL 344 PENNTVQASSSTVTAVALOPNTLSPLFVLNMEGSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMEGSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMEGSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMDSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMDSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMNASVSARTVSGLTV 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMDSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMDSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMDSVSARVNVGHKL 344 PENNTVQASSTVTAVALOPNTLSPLFVLNMNASVSARTVSGLTV	**************************************
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Pig Cattle Human Black rockfish Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat	254 PFSPAAFSLFPOINNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPQA 254 PFSPAAFSLFPOINNMLYISVSAFTINSAAFVYNKAGALSLYITDDMIPQS 254 PFSPAFFLNGEGSMLYIGLSAFTANSASFVYNKAGTLSLKIDDMVPRS 254 PFSPAFFLNGEGSMLYIGLSAFTANSASFVYNKAGLSLVITDDMIPS 255 PFSAFFSLPOISNMLYIGLSAFTANSASFVYNKAGLSLVITDDMIPS 254 PFSPAFFSLPOISNMLYIGLSAFTANSASFVYNKAGLSLVITDDMIPS 254 PFSPIFSLPOINNLYIGVSSFTNSAGFYYNKAGALSLVITDDMIPS 254 PFSFIFSLPOINNLYIGVSSFTNSAGFYYNKAGALSLVITDDMIPS 254 PFSFIFSLPOINNLYIGVSSFTNSAGFYYNKAGALSLVITDDMIPS 255 PFSFIFSLPOINNLYIGUSSFTNSAGFYYNKAGALSLVITDDMIPS 256 PFSFTFSLPOINNLYIGUSSFTNSAGFYYNNAGALSLVITDDMIPS 264 PAUPYNNILYIGLSAFTNSAGFYYNNAGALSLVITDDMIPS 264 PAUPYNNILYIGUSSFTNSAGFYYNNAGALSLVITDDMIPS 265 PFSPTFSLPOINNLYIGUSSFTNSAGFYYNNAGALSLVITDDMIPS 266 PFSPTFSLPOINNLYIGUSSFTNSAGFYYNNAGALSLVITDDMIPS 266 PFSPTPSLPOINNLYIGUSSYFTNTAGLYYQKAGUNUTINGNIPKS 269 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYYQKAGUNUTINGNIPKS 269 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYYQKAGUNUTINGNIFKS 269 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYYQKAGUNUTINGNIFKS 269 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYVQKAGUNUTINGNIFKS 269 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYVQKAGUNUTINGNIFKS 269 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYVQKAGUNUTINGNIFKS 261 PFAPPALAFSDHIRWYIGUSSYFTNTAGLYVQKAGUNUTINGNIFKS 261 PFAPPALSTAFSTNTAGUSYVCKKUTUSSYTTNTAGUNVKAKU 261 PFSPTSTTAGSTVTAYATOPNTILSPLFULNLETSVSARVFVSGKKL 261 PFAPPUNTUGASSTATAATLANTKLAPLEVLNLDFSVSARVVVSKKL 261 PFSPTSTASSTATAATLANTKLAPLEVLNLDFSVSARVVVSKKL 261 PFSPTSTASSTATAATLANTKLAPLEVLNLBSVSARVVVSKKL 261 PFSPSSTAFSSTATAATLANTKLAPLEVLNLDSVSSARVVVSKKL 261 PFSPSSTAFSSTATAATLANTKLAPLEVLNLDSVSSARVVVSKKL 261 PFSPSSTAFSSTATAATLANTKLAPLEVLNNBSVSSARVVVSKKL 261 PFSPSSTAFSSTATAATLANTKLAPLEVLNNBSVSSARVVVSKKL 261 PFLNNTLGASSTVTAATOPNSLLSPLEVLNNBSVSSARVVSKKL 261 PFLNNTLGASSTVTAATOPNSLLSPLEVLNNBSVSSARVVSKKL 261 PFSPSSTAFNKLETOAPVVLPNSSLLPLEVLNNNASVSARTVSGLTVS 251 PFSPSSTAFNKLETOAPVVLPNSSLPLEVLNNSSVSSARTVSKKRL 251 PFSPSSTAFNKLETOAPVVLPNSSLPLEVLNNBSVSSARTVSKRL 251 PFSPSSARTNATATANTAFSSARTVSKAFSTAFSFSTAFSTAFSSARTVSKAFSSARTVSKRL 2551 NKRAFTNATATLANTKLEPLEVLNNSSVSSARTVSKRL 2551	****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      ****      *****      ******
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Nousy rat Buck rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 1 Commel catfish Norway rat House mouse Dog Pig Cattle	254 PFSPAAFSLFPONNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPQA 254 PFSPAAFSLFPONNMLYISVSAFTINSAAFVYNKAGALSLYITDDMIPQS 254 PFSPAFFLNG©GSMLYIGLSAFTANSASFVYNKAGALSLYITDDMIPGS 255 PFSAFFSLPOUSMLYIGLSAFTANSASFVYNKAGALSLVITDDMIPGS 256 PFSAFFSLPOUSMLYIGLSAFTANSASFVYNKAGALSLVITDDMIPGS 256 PFSAFFSLPOUSMLYIGLSAFTANSASFVYNKAGALSLVITDDMIPGS 256 PFSFSFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 256 PFSFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFPFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFPFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFPFNALPPUNNHYMCMISSYFFNISAGFYYNKAGALSLVITDDMIFGS 266 PFAPPALAFSDDINHLYIGUSSFTINSAGFYYNKAGALSLVITDDMIFGS 266 PFAPPALAFSDDINHYYLGISSYFFNISAGFYYNKAGALSLVITDDMIFGS 266 PFAPPALAFSDDINHYYLGISSYFFNISAGFYYNKAGALSLVITUDMIFGS 267 PFAPPALFFYNNNYHCMISSYFFNISAGFYYNKAGALNUTLGOLLAND 268 PFFPFNALPPUNNHYMCMISSYFFNISAGFYYNKAGALNUTLGOLLAND 269 PFAPPALFFYNNYHYLGISSYFFNISGLVYQEAGUNUTLGOLLAND 269 PFAPPALAFSDDIRWYLGISSYFFNISGLVYQEAGUNUTLGOLLAND 261 PFAPPALAFSDDIRWYLGISSYFFNISGLVYQEAGUNUTLGNMIFKS 269 PFAPPALAFSDDIRWYLGISSYFFNISGLVYQEAGUNUTLGNMIFKS 269 PFAPPANAFYNYNATOPNILSPLFILMLETSVSARVFVSGKAL 244 I ERNKVIVQAKSSLTAYAILANTKLAPLEVLINLDFSVSARVVVGKKL 244 I FENNVTQASSSTAYAILANTKLAPLEVLINLDFSVSARVVVGKKL 245 PFENNVTQASSSTAYAILANTKLAPLEVLINLDFSVSARVVVGKKL 246 I PFAPPALAFSSLTAYAILANTKLAPLEVLINLBSVSARVVVGKKL 246 I PFAPPALFFINITUDASSTAYAILANTKLAPLEVLINLBSVSARVVVGKKL 246 I PFAPPALFFINITUDASSTAYAALAPLEVLANBSVSARVVVGKKL 247 I FENNVTQASSSTAYAALOPNILSPLEVLINLBSVSARVVVGKKL 248 I FENNVTQASSSTAYAALAPHTLANTKLAPLEVLINLBSVSARVVVGKKL 249 I FENNVTQASSSTAYAALAPHTLSPLEVLANBSVSARVVGKKL 240 I FENNVTQASSSTAYAALOPNTLSPLEVLANBSVSARVVGKKL 241 I EENNVTQASSTAYAALOPNTLSPLEVLANBSVSARVVGKKL 254 I (PSGLSISSTVTAAACPAVLPNSLAPLEVLANNASVSARLYVGKKL 254 I (PSGLSISSTVTAACPAVLPNSLAPLEVLANNASVSARLYVGKKL 255 I PFAPPLAFFILTDAPAVLPNSLAPLEVLANTNASVSARLYVGKKL 256 I PFAPPLAFFILTDAPAVLPNSLAPLEVLANTNASSTARLYVGKKL 256 I PFAPPLAFFILTDAPAVLPNSLAPLEVLANSABSVSARLYVGKKK	************************************
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Notway rat House mouse Dog Fig Cattle Human Black rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Notway rat House mouse Dog Pig	<pre>254 [PFSPAAFSLFPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA; 254 [PFSPAAFSLFPOINNMLYTSVSAFTINSAAFVYNKAGALSLYTTDDMIPQS; 254 [PFSPAFFLINDGCSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS; 254 [PFSPAFFLINDGCSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS; 254 [PFSPAFFSLPOCISMLYTGLSAFTANSASFVYNKAGALSLYTDDMIPS; 254 [PFSPTFSLPOQNNMLYTGMSAFTINSAGFVYNNAGALSLYTDDMIPS; 254 [PFSPTFSLPOQNNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPS; 254 [PFSPTFSLPOQNNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPS; 256 [PFSPTFSLPOQNNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPS; 266 [PFSPTFSLPOQNNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPS; 266 [PFSPTFSLPOQNNMLYTGVSFTNSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGFVYNNAGALSLYTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGFVYNNAGALSLYTTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGFVYNNAGALSLYTTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGLYQGAGALLMITTDDMIPS; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGLYQGAGALLMITTDDMIPK; 266 [PFTPFAALDEDSNMYTGLSSTFNSAGLYQGAGALLMITTDDMIPK; 267 [PFTPFAALDEDSNMYTGLSSTFNSAGLYQGAGALLMITTDDMIPK; 268 [PFNFTQAGSVTAAAICDNSTLSELFILMIETSVSAGVYTSGML]; 264 [PENNTYQASSLTAVAILANTKLAPLEVLMIDFSVSAAVNVGNKL]; 264 [PENNTYQASSLTAVAILANTKLAPLEVLMIESVSAAVTYGVKL]; 264 [PENNTYQASSTVTAVAICPNSTLSPLEVLMIESVSAAVTYGVKL]; 264 [PENNTYQASSTVTAVAICPNSTLSPLEVLMIESVSAAVTYGVKL]; 264 [PENNTYQASSTVTAVAICPNSLSLEPLEVLMIPSVSAAVNVGNKL]; 264 [PENNTYQASSTVTAVAICPNSLSLEPLEVLMI</pre>	*** *** *** *** *** *** *** *** *** **
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Commol catfish Nousy rat Buck rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 1 Commel catfish Norway rat House mouse Dog Pig Cattle	254 PFSPAAFSLFPONNMLYISVSAFTINSAAFVYNTAGALSLYITDDMIPQA 254 PFSPAAFSLFPONNMLYISVSAFTINSAAFVYNKAGALSLYITDDMIPQS 254 PFSPAFFLNG©GSMLYIGLSAFTANSASFVYNKAGALSLYITDDMIPGS 255 PFSAFFSLPOUSMLYIGLSAFTANSASFVYNKAGALSLVITDDMIPGS 256 PFSAFFSLPOUSMLYIGLSAFTANSASFVYNKAGALSLVITDDMIPGS 256 PFSAFFSLPOUSMLYIGLSAFTANSASFVYNKAGALSLVITDDMIPGS 256 PFSFSFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 256 PFSFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFPFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFPFSLPOUSMLYIGUSSFTINSAGFYYNKAGALSLVITDDMIPGS 266 PFFPFNALPPUNNHYMCMISSYFFNISAGFYYNKAGALSLVITDDMIFGS 266 PFAPPALAFSDDINHLYIGUSSFTINSAGFYYNKAGALSLVITDDMIFGS 266 PFAPPALAFSDDINHYYLGISSYFFNISAGFYYNKAGALSLVITDDMIFGS 266 PFAPPALAFSDDINHYYLGISSYFFNISAGFYYNKAGALSLVITUDMIFGS 267 PFAPPALFFYNNNYHCMISSYFFNISAGFYYNKAGALNUTLGOLLAND 268 PFFPFNALPPUNNHYMCMISSYFFNISAGFYYNKAGALNUTLGOLLAND 269 PFAPPALFFYNNYHYLGISSYFFNISGLVYQEAGUNUTLGOLLAND 269 PFAPPALAFSDDIRWYLGISSYFFNISGLVYQEAGUNUTLGOLLAND 261 PFAPPALAFSDDIRWYLGISSYFFNISGLVYQEAGUNUTLGNMIFKS 269 PFAPPALAFSDDIRWYLGISSYFFNISGLVYQEAGUNUTLGNMIFKS 269 PFAPPANAFYNYNATOPNILSPLFILMLETSVSARVFVSGKAL 244 I ERNKVIVQAKSSLTAYAILANTKLAPLEVLINLDFSVSARVVVGKKL 244 I FENNVTQASSSTAYAILANTKLAPLEVLINLDFSVSARVVVGKKL 245 PFENNVTQASSSTAYAILANTKLAPLEVLINLDFSVSARVVVGKKL 246 I PFAPPALAFSSLTAYAILANTKLAPLEVLINLBSVSARVVVGKKL 246 I PFAPPALFFINITUDASSTAYAILANTKLAPLEVLINLBSVSARVVVGKKL 246 I PFAPPALFFINITUDASSTAYAALAPLEVLANBSVSARVVVGKKL 247 I FENNVTQASSSTAYAALOPNILSPLEVLINLBSVSARVVVGKKL 248 I FENNVTQASSSTAYAALAPHTLANTKLAPLEVLINLBSVSARVVVGKKL 249 I FENNVTQASSSTAYAALAPHTLSPLEVLANBSVSARVVGKKL 240 I FENNVTQASSSTAYAALOPNTLSPLEVLANBSVSARVVGKKL 241 I EENNVTQASSTAYAALOPNTLSPLEVLANBSVSARVVGKKL 254 I (PSGLSISSTVTAAACPAVLPNSLAPLEVLANNASVSARLYVGKKL 254 I (PSGLSISSTVTAACPAVLPNSLAPLEVLANNASVSARLYVGKKL 255 I PFAPPLAFFILTDAPAVLPNSLAPLEVLANTNASVSARLYVGKKL 256 I PFAPPLAFFILTDAPAVLPNSLAPLEVLANTNASSTARLYVGKKL 256 I PFAPPLAFFILTDAPAVLPNSLAPLEVLANSABSVSARLYVGKKK	*** **********************************
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Commol catfish Nousy rat Buck rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 1 Commel catfish Norway rat House mouse Dog Pig Cattle	<ul> <li>254 PEPEPARAPSLEPCONNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPOA.</li> <li>254 PEPEPARE LEPCNDDMLYMCMSAYSVNSAAFVYNKAGALSLYTTDDMIPOS.</li> <li>254 PEPEPARE LENGECSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS.</li> <li>254 PEPERE LENGECSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS.</li> <li>254 PEPERE LENGECSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS.</li> <li>254 PEPERLENGECSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS.</li> <li>254 PEPERLENGECSMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPRS.</li> <li>254 PEPERLENGECSMLYTGLSAFTANSASFVYNKAGTLSLKITDDMIPRS.</li> <li>254 PEPERLENGECSMLYTGLSAFTINSAGFVYNNAGALSLYTDDMIPRS.</li> <li>254 PEPERLENGEDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPRS.</li> <li>256 PEPERLENGEDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS.</li> <li>266 PEPERLENGEDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS.</li> <li>266 PEPERLENGENSMATGLSAFTINSAGFVYNNAGALSLYTDDMIPS.</li> <li>266 PEPERLENGENSMATGLSAFTINSAGFVYNNAGLSLIKMTLGGLLSIM.</li> <li>266 PERPERLENGENSMATGLSAFTINSAGFVYNNAGLSLIKMTTAGGLSLISM.</li> <li>266 PERPERLENGENSMATGLSAFTINSAGFVYNNAGLUSLITTDMIPS.</li> <li>266 PERPERLENGENSMATGLSUFFNITAGLYVQCAGULMITTDMIPS.</li> <li>266 PERPERLENGENTYNTGLSUFFNITAGLYVQCAGULMITTDMIPS.</li> <li>266 PERPERLENGENTYNTAGLSUFFNITAGLYVQCAGULMITTDMIPS.</li> <li>267 PERPERLENGENTYTTAYATOPNITTLSELFULMLETSVSARVESMLIK.</li> <li>268 PERPERVERFARADERVYLGUSSTYFNITAGLYVQCAGULMITTDMIPS.</li> <li>264 PERVERVENSKSTAVATIOPNITTLSELFULMLETSVSARVESMLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMLETSVSARVESMLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMLETSVSARVESMLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMLETSVSARVESMLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMLBESVSARVESKLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMLBESVSARVESKLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMLBESVSARVESKLIK.</li> <li>264 PERVERVENSKSTAVATIOPNITLSELFULMABESVSARVESKLIK.</li> <li>265 PERVERVENSKSTAVATATOPNITLSELFULMABESVSARVESKLIK</li></ul>	*** *** *** *** *** *** *** *** *** **
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Fig Cattle Human Black rockfish Olive flounder Atlantic cod A Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat House mouse Dog Fig Cattle Human Black rockfish Olive flounder	<pre>254 [PFSPAAFSLEPCNINMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA; 254 [PFSPAAFSLEPCNIDMLYMMSAYSVNSAAFVYNKAGALSLYTTDDMIPQ3; 254 [PFSPAFFLENGESMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPR3; 254 [PFSPAFFLENGESMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPR3; 254 [PFSPAFFLENGESMLYTGLSAFTANSASFVYNKAGTLSLKITDDWPR3; 254 [PFSPTFSLPQVNNLYTGNSAFTINSAGFVYNNAGALSLYTDDMIPP3; 254 [PFSPTFSLPQVNNLYTGNSAFTINSAGFVYNNAGALSLYTDDMIPP3; 254 [PFSPTFSLPQDNNLYTGVSFTNSAGFVYNNAGALSLYTDDMIPP3; 256 [PFSPTFSLPQDNNLYTGVSFTNSAGFVYNNAGALSLYTDDMIPP3; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPP3; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS]; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS]; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS]; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS]; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS]; 266 [PFTPFADADDSNMYTGLSAFTINSAGFVYNNAGALSLYTDDMIPS]; 266 [PFTPFADADDSNMYTGLSISYFNNTAGLYQGAGALNLTITDDMIPS]; 266 [PFTPFADADDSNMYTGLSISYFNNTAGLYQGAGALNLTITDDMIPS]; 266 [PFTPAADADPHDPHMYLGJISYFPNTAGLYQGAGALNLTITDDMIPK3; 374 [PENNTYLQATSTYTAYATGNDTTLSELFILMLETSVSAQVFISGMNJ]; 344 [PENNTYLQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYLQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNVGNL]; 344 [PENNTYQASSTYTAYATGPNTLSELFILMLDFSVSARVNGNL]; 345 [PFSDVTLDYQAFYLPAJGPNTLSELFILMLDFSVSARVNGNL]; 346 [PENNTYQASSTYTAYATGPNTLSELFILMLNSVSARLYNGSLT]; 346 [PENNTYQASSTYTAYATGPNTLSELFILSTLMNSVSARLYNGSLT]; 355 [VHSSGLDIFYLDTQAFYLPAJGPNTLSELFILMLNSVSARLYNGSLT]; 354 [PFSGLJFTPTLDTQAFAVLPNSLSLELFLEMLNINLSVVGRKSRL]; 355 [VHSSGLDIFYLDTQAFAVLPNSLSLEPSLENGAHTNSSISVGRKSRL]; 355 [VHSSGLDIFYLDTQAFAVLPNSLSLEPSLENGANINLSVVGRKSRL]; 356 [PFSGLJFTPTLDTQAFAVLPNSLSLEPSLENGANINLSVVGRKSRL]; 357 [VHSGLLFYPLDTQA</pre>	*********************************
Olive flounder Atlantic cod A Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat Dog Dog Cattle Human Black rockfish Olive flounder Atlantic cod B Ayu Rainbow trout 1 Rainbow trout 2 Common carp Channel catfish Norway rat Bog Cattle Human Black rockfish Olive flounder Aug Cattle Human	254 PFSPAAFSLFPOINNMLYTSVSAFTINSAAFVYNTAGALSLYTTDDMIPQA. 254 PFSPAAFSLFPOINNMLYTSVSAFTINSAAFVYNKAGALSLYTDDMIPQS. 254 PFSPAFFLNGGSMLYTGLSAFTANSASFVYNKAGALSLYTDDMIPGS. 254 PFSPAFFLNGGSMLYTGLSAFTANSASFVYNKAGALSLYTDDMIPGS. 254 PFSPAFFSLPQCSNMLYTGLSAFTANSASFVYNKAGALSLYTDDMIPGS. 254 PFSPAFFSLPQCNNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPGS. 254 PFSFTFSLPQCNNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPGS. 254 PFSFTFSLPQCNNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPGS. 254 PFSFTFSLPQCNNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPGS. 254 PFSFTFSLPQCNNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPGS. 255 PFSFTFSLPQCNNMLYTGNSFTINSAGFVYNKAGALSLYTDDMIPGS. 266 PAPPTPSLPQCNNMLYTGNSFTINSAGFYYNKAGALSLYTDDMIPGS. 266 PFTPSPAFPLANKMVCGISDYFNINAGLYQGSGTLKTINAQULSLYTDMIPGS. 266 PFPPANALPPONNNLYLGISYFFNIAGLYQGAGULMITTNNNFIPKS. 267 PFAPPALAFSDHRMYLGISSYFFNIAGLYQGAGULMITTNNNFIPKS. 268 PFAPPALAFSDHRMYLGISSYFFNIAGLYQGAGULMITTNNFIPKS. 269 PFAPPALAFSDHRMYLGISSYFFNIAGLYQGAGULMITTNNFIPKS. 269 PFAPPALAFSDHRMYLGISSYFFNIAGLYQGAGULMITTNNFIPKS. 269 PFAPPALAFSDHRMYLGISSYFFNIAGLYQEAGULMITLRDMIPKS. 269 PFAPPALAFSDHRMYLGISSYFFNIAGLYQEAGULMITLRDMIPKS. 269 PFAPPALAFSDHRMYLGISSYFFNIAGLYQEAGULMITLRDMIPKS. 269 PFAPPUAFSTDHRMYLGISSYFFNIAGLYVGAGAULMITLRDMIPKS. 269 PFAPPUAFSTDHRMYLGISSYFFNIAGLYVGAGAULMITLRDMIPKS. 269 PFAPPUAFSTDHRMYLGISSYFFNIAGLYVGAGAULMITLRDMIPKS. 269 PFAPPUAFSTDHRMYLGISSYFFNIAGLYVGAGAULMITLRDMIPKS. 269 PFAPUAFSTDHRMYLGISSYFFNIAGLYVGAGAULMITLRDMIPKS. 261 PFAPUAFASTDATAATOPNTTLSPLEVLNUDFSVSARVVVGKKLJ. 344 PEPNNTUQASSTVTAYATOPNTLSPLEVLNUBSVSARVVVGKKLJ. 344 PEPNNTUQASSTVTAYATOPNTLSPLEVLNUBSVSARVVVGKKLJ. 344 PEPNNTUQASSTVTAYATOPNTLSPLEVLNUBSVSARVVVGKKLJ. 344 PEPNNTUQASSTVTAYATOPNTLSPLEVLNUBSVSARVVGKKLJ. 344 PEPNNTUQASSTVTAFATOPNTLSPLEVLNUBSVSARVVGKKLJ. 344 PEPNNTUQASSTVTAFATOPNTLSPLEVLNUBSVSARVVGKKLJ. 344 PEPNNTUQASSTVTAFATOPNTLSPLEVLNUBSVS	****         PIRLINTETFORF TFDEVAKRP FOLLMKLLVGTVKTEVIT         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVGTVKTEVIT         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVGTVKTEVIT         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVGTVKTEVIT         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVGLVKTVDEPITS         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVKTVDEFITS         341         SPIRLINTETFORT FIELSKRP FOLMMKLLVKTVDEFITS         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVKTVKEFTIS         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVKTVKEFTIS         343         SPIRLINTETFORT FIELSKRP FOLMMKLLVKTVKEFTIS         345         SKRLITTEFORT FIELSKRP FOLMKLLVETVKERFINS         SIFELSTITEFORT FIELSKRP FOLMOLDLISABEVPHILS         SIFELSTITEFORT FIELSKRP FOLMOLDLISABEVERLY         SIFELSTITEFORT FIELSKRP FOLMOLDLISABEVPHILS         SIFELSTITEFORT FIELSKRP FOLMOLDLISABEVPHILS
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Fig. 2. Comparison between the black rockfish BPI/LBP amino acid sequence and other known BPI or BPI/LBP sequences. The amino acids identical to the black rockfish sequence are indicated by an asterisk (\*), and the absent amino acids are indicated by dashes (-). The accession numbers of BPI or BPI/LBP for the creation of multiple alignment used are described Fig. 4. The putative signal peptide is indicated black box. Identities of between black rockfish BPI/LBP and other BPI/LBP or BPI is indicated box. Central domain and N-terminal domain and C-terminal domain is indicated gray and underline, respectively. 231 and BPI2 (BPI/LBP/CETP C-terminal domain) between amino acid residues 246 and 449 were identified by the SMART program (Fig. 1). The mature Black rockfish BPI/LBP was predicted by comparing the amino acid sequence with that of other mammalian LBP and BPI. The mature Black rockfish BPI/LBP was also separated into the N-terminal domain (residues 8-231) and C-terminal domain (residues 246-449). The proline-rich central domain (resi dues 228-248) was located between the N-terminal and C-terminal domains (Fig. 1). The two domains form the three structural elements, the N-terminal barrel (barrel-N), a central  $\beta$ -sheet, and the C-terminal barrel (barrel-C) (Beamer *et al.*, 1998).

The deduced amino acid sequence of black rockfish BPI/LBP had a 79.7% identity with that of Olive flounder LBP/BPI, 75.5% with Ayu BPI/LBP, 75.2% with Rainbow trout LBP/BPI-1, 74.8% with Rainbow trout LBP/BPI-2, 72% with Common carp BPI/LBP, 68.2% with Channel catfish BPI, 63.2% with Atlantic cod BPI/LBP-A and B, 38.7% with Dog BPI, 35.7% with

Cattle BPI and Human, 34.1% with Pig BPI, 29.7% Norway rat BPI, and 27.5% with House mouse BPI. (Fig. 2). Furthermore, multiple sequence alignment revealed that the LPS binding domain was well conserved.

Moreover, the LPS-binding domain included in barrel-N was aligned with those of olive flounder LBP/BPI, ayu BPI/LBP, Atlantic cod BPI/LBP-a and -b, rainbow trout BPI/LBP-1 and -2, channel catfish BPI, common carp BPI/LBP, house mouse BPI and LBP, Norway rat BPI and LBP, dog BPI and LBP, pig BPI and LBP, cattle BPI and LBP, and human BPI and LBP (Fig. 3). When LPS-binding domain of black rockfish BPI/LBP was compared with that of other teleosts, that of black rockfish BPI/LBP showed the highest identity (80.0%) to that of olive flounder LBP/BPI. The identities to that of human BPI and LBP were 33.8 and 35.7%, to that of black rockfish BPI/LBP, respectively (Fig. 3).

LBP and BPI are distinguished from PLTP and CETP because they have the function of binding LPS with high affinity (Inagawa *et al.*, 2002). Recent analyses

Black rockfish	GKORVSPIGKVKYSLSNMOIVDVGLPKSALDLVPGTGVKLSIGNAFLRMHGNWRVKYLRIIKDSG	Identities (%)
Olive flounder	GKERVPPIGKVEYSLSHMQIVKLGLPKSAVDLVPGTGIKLSISNAYISLHGNWRVKYLRIIKDSG	80.0
	GKERVITISKVETSESEVIJU VELGERKSVEGEVEGTGVELSIGNAFINER VRTERTERDSG GSAKVAPIGKVTYSLTGMQIVELGERKSVEGEVEGTGVELSIGNAFENERGRAVRITERTERDSG	73.8
Ayu		
Atlantic cod A	GKERVKPIGKVRYSVTEMRVVKLGLPSSAIGLVPGTGFSLTITNAFLSLHGRWRVKYLRFIKDRG	66.1
Atlantic cod B	GKERVKPIGKVRYSVTEMRVVKLGLPSSAIGLVPGTGFSLTITNAFLSLHGRWRVKYLRFIKDRG	66.1
Rainbow trout 1	GTERVAPIGKVKYSLTGITIVNLGLPYSALALVPDTGISLSITNAFISLHGNWKIRYLSFIKDSG	64.6
Rainbow trout 2	${\tt GTEKVPPIGKVKYSLTGMTIVNLGLPKSALVLMPGTGVRLAITNAFINLHGNWRVRYFRFIQDRG}$	66.1
Channel catfish	GSEKVSPIGKVSYSLTGIQILDLGLPKSAVGLVPGTGVSLSIGDAYINLHGNWRVKYLRIIKDSG	73.8
Common carp	GTEKVDPIGKVQYSFTGMQIVNLGLPKSALVLVPDTGVMLSIGNAYINLHGNWRVKYLRIIKDSG	73.8
House mouse BPI	GVFKIKHLGKGSYEFYSMAVDGFHIPNPKIEMLPSDGLRVFIKDASIKINGKWMSR-KNFLKAGG	22.4
Norway rat BPI	GDFKIKHLGKGTYEFYSMAVEGFHIPDPQIKLLPSDGLQLSITSASIKISGRWKYR-KNILKASG	30.7
Dog BPI	GSFKVKHLGKGHYSFYSMVIRGFQLPSSQIKLVPNKGLDLSIRNANIKISGKWKAR-KNFIKTSG	40.3
Pig BPI	GSFKIKYFGKGRYNFYSMVVREFKLPTSQIRLSPDRGLDLSIKDASVKISGKWKAQ-KNFIKASG	32.2
Cattle BPI	GNFKIKYLGKGQYSFFSMVIQGFNLPNSQIRPLPDKGLDLSIRDASIKIRGKWKAR-KNFIKLGG	29.2
Human BPI	DSFKIKHLGKGHYSFYSMDIREFQLPSSQISMVPNVGLKFSISNANIKISGKWKAQ-KRFLKMSG	33.8
House mouse LBP	GDFKIKAVGRGQYEFHSLEIQNCELRGSSLKLLPGQGLSLAISDSSIGVRGKWKVR-KSFLKLHG	28.3
Norway rat LBP	GDFKIKAVGRGQYEFHSLEIQSCQLRGSSLKPLPGRGLSLSISDSSISVRGKWKVR-RSFVKLHG	28.3
Dog LBP	GDFKIKPIGRGHYDFHSLSVHSCELRGSALTPLPSQGLSLTISDSFIRVQGEWKAR-KAFVKLQG	30.1
Pig LBP	GDFKIKYMGRGHYEFHSLDIHSCELLGSTLTPLPGQGLYLAISDSSIRVKGKWKVR-KGILKLDG	28.3
Cattle LBP	GDVR1KHFGSVDYRFHSLN1QSCKLLGSALKLLPNQGLHFS1SDSF1QVTGDWKVR-KR1LRLDG	33.8
Human LBP	GDLRIPHVGRGRYEFHSLNIHSCELLHSALRPVPGQGLSLSISDSSIRVQGRWKVR-KSFFKLQG	35.7

Fig. 3. Alignment of the predicted LPS-binding domain of the black rockfish BPI/LBP with that of other species. Identical amino acid residues are indicated by asterisks (\*). Dots (·) indicate the black rockfish BPI/LBP. The positively charged amino acid residues (K or R) conserved in BPI/LBP are indicated by shaded regions. The number at the end of each sequence represents amino acid identities (%) to that of the black rockfish LBP/BPI.

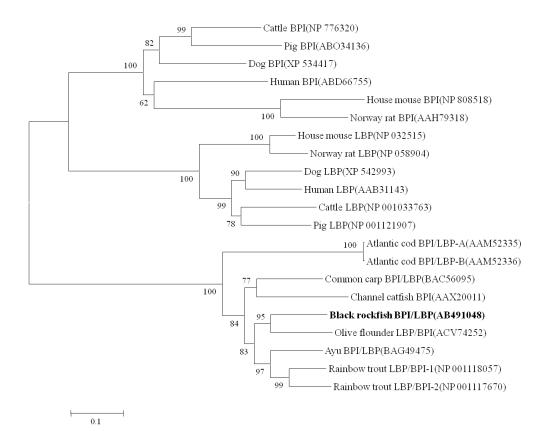


Fig. 4. Neighbor-joining tree of BPI/LBP constructed with Mega4. The bootstrap confidence values shown at the nodes of the tree are based on 2000 bootstrap 2000 replications (DDBJ/EMBL/GenBank Accession numbers: Black rockfish BPI/LBP (AB491048), Olive flounder LBP/BPI (ACV74252), Ayu BPI/LBP (BAG49475), Rainbow trout LBP/BPI-1 (NP 001118057), Rainbow trout LBP/BPI-2 (NP 001117670), Channel catfish BPI (AAX20011), Common carp BPI/LBP (BAC56095), Atlantic cod BPI/LBP-A(AAM52335), Atlantic cod BPI/LBP-B (AAM52336), Cattle BPI (NP 776320), Pig BPI (ABO34136), Dog BPI (XP 534417), Human BPI (ABD66755), House mouse BPI (NP 808518), Norway rat BPI (AAH79318), House mouse LBP (NP 032515), Norway rat LBP(NP 058904), Dog LBP (XP 542993), Human LBP (AAB31143), Cattle LBP (NP 0011033763), Pig LBP(NP 001121907)).

suggest that conserved positive charge amino acids in the N-terminal barrel of mammal LBP and BPI (lysine 42, 48, 92, 95, and 99 in human BPI) were involved in binding the anionic portion of lipid A (Beame *et al.*, 1998). As these residues are clustered at the tip of the NH<sub>2</sub>-terminal domain, they may cause electrostatic interactions with negatively charged groups of LPS (Beame *et al.*, 1998; Lamping *et al.*, 1996). As shown in Fig. 3, basic amino acid residues in black rockfish BPI/LBP (lysine or arginine 42, 48, 92, 94, and 100 in BR-BPI/LBP) were well conserved at a similar position (Fig. 3). These data suggest that black rockfish BPI/LBP proteins might bind LPS, and thus may have a pivotal role in the innate immune system in fish.

The phylogenetic analysis indicated that the BPI and/or LBP sequences from mammals and teleosts

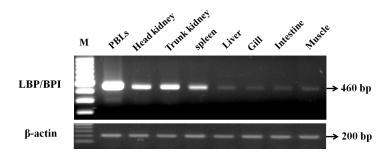


Fig. 5. Detection of the black rockfish BPI/LBP mRNA levels and β-actin from various tissues of healthy black rockfish via RT-PCR. The marker indicates a 100-bp ladder marker.

segregated into two separate clusters and is closest to that of olive flounder LBP/BPI among the examined species. Moreover, all the mammalian BPI and LBP sequences clustered into distinct clades, This grouping was well-supported by bootstrapping (Fig. 4). The majority of fish BPI/LBP sequences clustered according to the species of origin. This result indicates that BPI and LBP in mammals are molecules of common origin, and duplication of BPI and LBP genes in mammals occurred after divergence from teleosts.

The expression of the BPI/LBP gene in the black rockfish tissues was detected via RT-PCR. The BPI/LBP gene was expressed in all the tissues of the black rockfish. Especially, the expression was found to be predominantly in the PBLs, and less dominantly in the head kidney, trunk kidney, spleen, and least in the liver, gill, intestine,

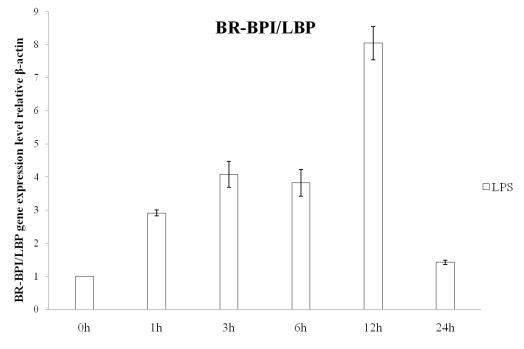


Fig. 6. Quantitative real-time PCR analysis of the BPI/LBP expression in black rockfish leukocytes stimulated with LPS (50µg/ml) at 0, 1, 3, 6, 12, and 24 h.

muscle after 25 cycles of PCR (Fig. 5). Some kind of leukocytes are probably the main source of BPI/LBP mRNA in blood as well. It is thus possible that leukocytes may be the main source of BPI/LBP in the spleen, heart, gills, and liver, as these organs are blood filled. This pattern was also similar to that found in Atlantic cod (Stenvik *et al.*, 2004) and large yellow croaker (Huang *et al.*, 2008), which inhabit seawater. However common carp (Kono and Sakai, 2003) and channel catfish (Xu *et al.*, 2005), which inhabit freshwater, was a contrast to black rockfish BPI/LBP expression pattern. In the latter BPI/LBP mRNA expression was high in the gill, intestine, and skin. Further work is necessary to reveal the reason why the different fish species have distinct gene expression patterns of BPI/LBP.

Black rockfish peripheral blood leukocytes (PBLs) were treated with LPS and the expression level of BR-BPI/LBP was examined. The LPS treated PBLs showed a marked increase in BR-BPI/LBP expression. Especially, the BPI/LBP levels seemed to reach a peak in expression at 12 h post-LPS stimulation, and decreased at 24 h (Fig. 6). Stenvik et al. (2004) repoted that the possible lack of the main components of the mammalian LPS-recognition system, including LBP, CD14, and TLR4, was speculated to explain the low LPS sensitivity of fish. Moreover, the CD14 and TLR4 orthologs have not been identified in black rockfish and the other teleosts. Even though, the black rockfish BPI/LBP mRNA expression level seemed like to lower tendency, these results indicate that the increased BR-BPI/LBP mRNA level is an early and short-lived response to stimulation by LPS. Through further investigation, we substantiate either the possibility of LPS-recognition system in black rockfish, or the presence of CD14 and TLR4 orthologs.

The study of innate immunity of fish has been hampered by a lack of molecular tools like gene probes and specific antibodies. Thus, BPI/LBP could become useful as a molecular tool for the study of innate immune mechanisms of fish. This information could help explain the immune system in fish, and might also illuminate the role of the innate immune system in other species.

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