

Expressed sequence tags analysis of immune-relevant genes in rock bream *Oplegnathus fasciatus* gill stimulated with LPS

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We constructed a rock bream (*Oplegnathus fasciatus*) gill cDNA library and a total of 1450 expressed sequence tag (EST) clones were generated. Gene annotation procedures and homology searches of the sequenced ESTs were locally done by BLASTX for amino acid similarity comparisons. Of the 1450 EST clones, 1022 EST clones showed significant homology to previously described genes while 428 ESTs were unidentified, and 259 clones were hypothetical, or unnamed proteins. Encoding 313 different sequences were identified as putative bio-defense genes or genes associated with immune response.

Key words : Expressed sequence tags, LPS, Rock Bream *Oplegnathus fasciatus*, Gill

Rock bream, *Oplegnathus fasciatus*, belongs to the Oplegnathidae family and inhabits the coastal rocky-shores of Korea, Japan, Taiwan, and Hawaii (Nakabo, 2002). This species has attracted great interest among Korean fish farmers due to its high market value and consumer demand. The total production of this species, however, is not satisfactory compared to other commercially important fishes in Korea. The RSIV disease has been the major culprit for the mass mortality of rock bream in Korea (Sohn *et al.*, 2000; Jung and Oh, 2000).

Fish gills are complex and multi-functional tissues. The gill epithelium consists of several cell types including pavement cells, chloride cells, mucous cells,

and undifferentiated cells (Laurent, 1984). These cells cooperate to regulate homeostasis in the gills. Fish mucus secreted from the mucous cells covers and separates the gill epithelium from the external environment and is involved in various events including respiration, osmoregulation, and host defense against pathogenic microorganisms (Shephard, 1994).

Many molecular tools are available for characterizing the immune systems of mammals, especially human being and mice. With regard to fish, however, information on immune-related molecules is still limited. Recently, remarkable progress has been made in genetic technology with the discovery of rapid expressed sequence tag (EST) analysis, which allows the acquisition of massive DNA sequence information of many organisms, including several species of fish, in short

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time period (Zeng and Gong, 2002; Clark *et al.*, 2003; Rise *et al.*, 2004). Large scale EST analysis is also an efficient way for identification of genes and for analysis of their expression by means of expression profiling (Franco *et al.*, 1995; Azam *et al.*, 1996; Lee *et al.*, 2000). It offers a rapid and valuable first look at genes expressed in specific tissue types, under specific physiological conditions or during specific developmental stages. Currently, the number of fish-related ESTs in public databases is still small compared to mammalian sequences and there are relatively few tissue-specific cDNA libraries (Ton *et al.*, 2000). There has been an increasing public interest in this topic, but relatively little information is available about rock bream genes (Cho *et al.*, 2000). The lack of information may be one obstacle to the effective use of genetics in aiding both rock bream aquaculture and conservation activities. In this study, EST sequenced to screen for bio-defense or immune related genes in rock bream gill after treatment with LPS.

Materials and methods

Animals

Rock bream *O. fasciatus*, with an average weight of 150 grams were obtained from the Genetics and Breeding Research Center (Geoje). Forty-eight rock bream were injected intra-peritoneally (IP) with LPS (6 mg/kg). The fish were maintained in a tank with running artificial seawater at 23-25°C. At each sampling time (days 1, 3, 5, and 7), a total of six fish from each experimental group were sacrificed, gills were dissected out and quickly frozen in liquid nitrogen and stored at -80°C.

cDNA library construction

The cDNA library was constructed using mRNA prepared from LPS stimulated gills tissue of rock bream. The purified mRNAs taken from four different time periods were pooled to ensure complete coverage of expressed genes in the allotted timeframe and were used to construct a cDNA library. Libraries were constructed by using a modification of Maruyama and Sugano (1994). The synthesis of the first-strand cDNA from the purified mRNA and cDNA amplification were performed as described by Maruyama and Sugano (1994). The amplified PCR products were then digested with *Sfi*I, and cDNAs longer than 400 bp were ligated into *Dra*III-digested pCNS-D2 in an orientation-defined manner. The pCNS-D2 vector contains 5' *Eco*RI-*Dra*III-*Eco*RV-*Dra*III sites at multi cloning sites, which was achieved by modifying the pCNS vector (GenBank Accession no. AF416744). The ligated cDNA was then transformed into *E. coli* Top10F' (Invitrogen) by electroporation (Gene Pulser II, BioRad).

Single-pass sequencing of the 5'-termini of 1533 selected rock bream gill cDNA clones in plasmid form was performed using the ABI 3700 automatic DNA sequencer (PE Applied Biosystems) and the ABI prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

EST sequencing and analysis

Bioinformatic analysis was conducted to determine gene identities using Genetyx ver. 8.0 software (SDC software, Japan). Briefly, vector sequences were then removed and database search were limited to ESTs >400 bp in length. The sequence of each cDNA was compared

with 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 program BLASTX (Gish and David, 1993).

Results and Discussion

cDNA library construction

A cDNA library of 2×10^7 clones was constructed from the polyadenylated fraction of mRNA of rock

bream injected with LPS. The number of clones in the constructed cDNA library was deemed sufficient to cover the predominantly expressed mRNAs in rock bream gill stimulated with LPS .

A total of 1450 randomly selected clones were single-pass sequenced from the 5' end, resulting in the characterization of cDNA clones that were longer than 400 bp after elimination of vector sequence. The number of clones sequenced from the cDNA library and the average size of inserts are given in Table 1.

Table 1. Summary of sequences and clones represented

Numbers of ESTs (n)	1450
Putatively identified clones (n)	1022 (70%)
Hypothetical protein	208
Unnamed protein	51
Ribosomal protein	77
Immune related genes (n)	313
Other genes	373
Unidentified clones (n)	428 (30%)
Putatively identified different genes (n)	422
Total nucleotides (bp)	1,095,559
Average of sequenced length (bp)	756

EST sequencing and analysis

We performed single pass sequencing on 1450 randomly selected clones from a sea bass cDNA library targeting the 5'-terminus of each insert.

Of the 1450 clones, 1022 (70%) were identified as orthologs of known genes from rock bream or other organisms.

We performed a BLASTX search on all the sequences. 428 (30%) of the 1450 sequences resulted to unidentified, i.e., did not show any significant similarity to the sequences present in the public databases based on nucleotides or translated peptides.

Among the 1022 identified EST clones, 313 clones were immune related genes identified as homologous

to the previously reported genes from rock bream or other organisms. 373 different rock bream genes were identified including the 77 genes for ribosomal proteins that we have obtained (Table 1).

Gene annotation procedures and homology searches of the sequenced ESTs have been locally done by

BLASTX for amino acid similarity comparisons. The ESTs with significant similarities to known proteins were evaluated to determine if these remarkable similarities were caused by simple amino acid matches (Table 2).

Table 2. Rock bream LPS stimulated gill ESTs encoding for immune related genes.

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
06-E05	AB595025	Serotonin receptor 1F	<i>Monodelphis domestica</i>	XP_001379957.1	2.00E-50	49	901	1
07-B10	AB595034	Serotonin receptor 2B	<i>Tetraodon fluviatilis</i>	Q8UUG8.1	1.00E-94	81	906	1
01-H02	AB594989	Alkylated DNA repair protein alkB homolog 7	<i>Salmo sala</i>	NP_001133859.1	3.00E-55	81	879	1
09-G11	AB595066	Apolipoprotein C-I	<i>Salmo salar</i>	NP_001134834.1	3.00E-08	71	482	1
14-E10	AB595122	apolipoprotein E	<i>Oplegnathus fasciatus</i>	ACF21982.1	1.00E-20	94	938	16
08-B08	AB595052	Apolipoprotein O	<i>precursor Salmo salar</i>	ACI68171.1	2.00E-54	59	856	1
06-E04	AB595024	Apoptosis facilitator Bcl-2-like protein 14	<i>Danio rerio</i>	XP_001332133.1	2.00E-09	44	887	1
03-A04	AB594993	Apoptosis-associated speck-like protein containing a CARD	<i>Siniperca chuatsi</i>	ABR24505.1	3.00E-46	67	897	1
06-G01	AB595028	B-cell linker protein	<i>Salmo salar</i>	ACN10516.1	4.00E-65	60	929	1
14-H12	AB595127	beta-2 microglobulin	<i>Stizostedion vitreum</i>	AAW65850.1	3.00E-39	84	936	17
14-E09	AB595121	calcium homeostasis endoplasmic reticulum protein	<i>Taeniopygia guttata</i>	XP_002197407.1	6.00E-67	78	821	1
10-A09	AB595071	Casitas B-lineage lymphoma, isoform CRA_a	<i>Mus musculus</i>	EDL25568.1	3.00E-63	57	791	1
14-A03	AB595114	caspase 10	<i>Paralichthys olivaceus</i>	BAE98149.1	3.00E-76	70	804	1
01-F12	AB594987	Cathepsin H precursor	<i>Salmo salar</i>	ACI66895.1	1.00E-07	74	912	1
05-F04	AB595015	cathepsin L-like protein	<i>Lutjanus argentimaculatus</i>	ACO82386.1	1.00E-129	100	903	1
10-E06	AB595079	Cathepsin O	<i>Salmo salar</i>	NP_001134063.1	1.00E-88	75	840	1
09-F11	AB595065	cathepsin S s	<i>Lutjanus argentimaculatu</i>	ACO82388.1	1.00E-108	91	774	1
06-G04	AB595030	CC chemokine ligand 4	<i>Dicentrarchus labrax</i>	CAM32187.1	1.00E-29	74	979	1

Table 2 (Continued)

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
05-F05	AB595016	C-C chemokine receptor family-like	<i>Danio rerio</i>	XP_001346655.1	3.00E-32	65	912	1
08-A07	AB595050	C-C motif chemokine 20 precursor	<i>Salmo salar</i>	ACI66945.1	6.00E-12	76	770	1
02-E08	AB594992	CCAAT/enhancer binding protein delta2	<i>Oncorhynchus mykiss</i>	ABD84408.1	2.00E-57	62	866	1
06-E10	AB595026	CCAAT/enhancer-binding protein beta 2	<i>Epinephelus coioides</i>	ACL98106.1	2.00E-46	97	817	1
14-C08	AB595116	CD9 antigen	<i>Anoplopoma fimbria</i>	ACQ58340.1	0.002	86	968	3
13-H08	AB595112	collagen, type VI, alpha 1	<i>Danio rerio</i>	XP_698253.2	4.00E-14	66	926	1
07-B01	AB595033	collagen, type VI, alpha 2 <i>Danio rerio</i>	<i>Danio rerio</i>	XP_696164.2	2.00E-94	58	873	1
01-D09	AB594983	Complement C1q subcomponent subunit C precursor	<i>Salmo salar</i>	ACN11312.1	4.00E-48	47	685	1
13-A05	AB595101	C-type lectin 13	<i>Perca flavescens</i>	ACO82046.1	3.00E-35	62	710	10
14-H05	AB595124	C-type lectin 3	<i>Perca flavescens</i>	ACO82036.1	2.00E-34	60	717	5
06-G08	AB595031	C-type lectin 6	<i>Perca flavescens</i>	ACO82039.1	1.00E-27	65	396	2
06-B07	AB595021	cytochrome b	<i>Oplegnathus fasciatus</i>	YP_001974630.1	1.00E-139	86	909	4
12-D05	AB595093	cytochrome c oxidase I	<i>Micropterus dolomieu</i>	YP_002274334.1	2.00E-69	88	575	2
12-F10	AB595098	cytochrome c oxidase subunit I	<i>Oplegnathus fasciatus</i>	YP_001974620.1	2.00E-96	71	839	10
05-D11	AB595011	cytochrome c oxidase subunit II	<i>Oplegnathus fasciatus</i>	YP_001974621.1	1.00E-100	88	699	5
05-F10	AB595017	cytochrome c oxidase subunit III	<i>Oplegnathus fasciatus</i>	YP_001974624.1	1.00E-112	86	785	4
12-D11	AB595094	cytochrome c oxidase subunit VIa polypeptide 1	<i>Danio rerio</i>	CAP19431.1	9.00E-35	94	587	1
03-A11	AB594995	Cytochrome c oxidase subunit VIb isoform 1	<i>Osmerus mordax</i>	ACO10158.1	2.00E-44	100	530	1
12-C05	AB595090	Endothelial differentiation-related factor 1 homolog	<i>Salmo salar</i>	ACI67525.1	3.00E-71	90	893	1
10-B05	AB595073	ferredoxin-fold anticodon binding domain containing 1	<i>Danio rerio</i>	NP_001093489.1	7.00E-26	56	579	1
14-A05	AB595115	Ferritin, middle subunit	<i>Anoplopoma fimbria</i>	ACQ57875.1	6.00E-91	97	737	6
13-F10	AB595108	Fish-egg lectin	<i>Salmo salar</i>	ACN10420.1	6.00E-60	60	872	7
08-F01	AB595056	GATA-binding factor 3	<i>Salmo salar</i>	NP_001133239.1	2.00E-31	76	344	1
04-E09	AB595004	heat shock cognate 70	<i>Rhabdosargus sarba</i>	AAR97293.1	3.00E-25	94	607	1
07-C01	AB595035	heat shock cognate 71	<i>Paralichthys olivaceus</i>	ABG56391.1	5.00E-12	97	491	1
14-D07	AB595119	heat shock protein 90 beta	<i>Pagrus major</i>	AAP20179.1	8.00E-70	78	873	3

Table 2 (Continued)

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
12-H12	AB595100	Hemoglobin subunit alpha-A	<i>Seriola quinqueradiata</i>	Q9PVM4.3	1.00E-67	94	577	2
10-A02	AB595070	Hemoglobin subunit alpha-B	<i>Seriola quinqueradiata</i>	Q9PVM3.3	2.00E-60	86	568	1
06-A07	AB595020	Hemoglobin subunit beta-A	<i>Seriola quinqueradiata</i>	Q9PVM2.2	1.00E-70	93	928	1
01-E11	AB594985	heparin-binding EGF-like growth factor	<i>Danio rerio</i>	NP_001104696.1	4.00E-24	39	785	1
11-B06	AB595083	HSP-90	<i>Dicentrarchus labrax</i>	AAQ95586.1	1.00E-123	90	774	1
07-E04	AB595041	IgGfC-binding protein precursor	<i>Monodelphis domestica</i>	XP_001369345.1	1.00E-32	65	854	1
09-C01	AB595062	immunoglobulin heavy chain	<i>Siniperca chuatsi</i>	AAQ14863.1	4.00E-58	94	514	1
07-H03	AB595047	Immunoglobulin lambda-like polypeptide 1 precursor	<i>Anoplopoma fimbria</i>	ACQ58137.1	9.00E-66	68	820	1
01-A08	AB594979	immunoglobulin light chain	<i>Dicentrarchus labrax</i>	CAC16852.1	1.00E-11	97	714	4
06-A06	AB595019	inhibitor kappa B alpha	<i>Paralichthys olivaceus</i>	ABO38854.1	1.00E-125	86	938	1
10-B12	AB595074	inhibitor of nuclear factor kappa B kinase beta subunit	<i>Danio rerio</i>	NP_001116737.1	8.00E-74	77	805	1
11-H01	AB595088	interferon stimulated gene 15	<i>Sebastes schlegelii</i>	BAG72218.1	5.00E-51	82	768	1
01-E01	AB594984	IRF1	<i>Siniperca chuatsi</i>	AAV65042.1	7.00E-10	91	839	1
03-B02	AB594996	keratin 13	<i>Oncorhynchus mykiss</i>	NP_001117848.1	1.00E-78	75	886	1
06-G02	AB595029	keratin 15	<i>Epinephelus coioides</i>	ACL98116.1	1.00E-74	98	867	2
12-E05	AB595046	keratin 8	<i>Danio rerio</i>	XP_696736.2	3.00E-71	63	775	1
07-G09	AB595095	keratin 8	<i>Oreochromis mossambicus</i>	AAP22041.1	3.00E-07	96	936	1
14-C10	AB595117	keratin type I	<i>Acipenser baerii</i>	CAD38121.1	3.00E-40	64	892	1
07-E07	AB595043	Keratin, type I cytoskeletal 13	<i>Anoplopoma fimbria</i>	ACQ58237.1	1.00E-135	90	916	2
10-E10	AB595080	LDH-A	<i>Chromis punctipinnis</i>	AAP44526.1	1.00E-83	98	847	1
09-H09	AB595069	leukolectin protein	<i>Salmo salar</i>	NP_001152845.1	7.00E-08	54	370	1
01-B09	AB594982	lily-type lectin	<i>Platycephalus indicus</i>	BAE79274.1	4.00E-47	85	524	4
06-D05	AB595023	Lipopolysaccharide-induced tumor necrosis factor-alpha factor homolog	<i>Anoplopoma fimbria</i>	ACQ58646.1	2.00E-44	92	927	2
13-H05	AB595111	lymphocyte cytosolic protein 1 precursor	<i>Oncorhynchus mykiss</i>	CAM82803.1	1.00E-158	97	875	1
13-E08	AB595106	lysozyme	<i>Anopheles darlingi</i>	AAB61345.1	2.00E-04	48	907	1
03-H12	AB594998	macrophage myristoylated alanine-rich C kinase substrate	<i>Scophthalmus maximus</i>	ABJ98697.1	7.00E-09	62	934	1

Table 2 (Continued)

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
11-G12	AB595087	major histocompatibility class I receptor	<i>Stizostedion vitreum</i>	AAL11412.1	3.00E-66	75	868	3
05-D12	AB595012	major histocompatibility complex II gamma chains	<i>Sparus aurata</i>	CAP47207.1	5.00E-75	84	864	5
09-H07	AB595068	MHC class I alpha antigen	<i>Epinephelus akaara</i>	ABX80523.1	1.00E-103	80	848	1
09-H04	AB595067	MHC class II antigen alpha chain	<i>Larimichthys crocea</i>	ABV48906.1	3.00E-10	68	847	5
08-H05	AB595058	MHC class II antigen beta chain	<i>Larimichthys crocea</i>	ABV48908.1	1.00E-72	80	860	5
07-D01	AB595038	MHC class II antigen-associated invariant chain	<i>Lutjanus argentimaculatus</i>	ACO82381.1	1.00E-07	88	835	1
06-F05	AB595027	MHC class II protein	<i>Morone saxatilis</i>	AAA49378.1	1.00E-100	83	964	1
01-B07	AB594981	MHC II invariant chain	<i>Siniperca chuatsi</i>	AAS77256.1	1.00E-52	92	899	5
04-D08	AB595002	Mitogen-activated protein kinase organizer 1	<i>Salmo salar</i>	NP_001134557.1	1.00E-121	97	893	1
07-G07	AB595045	Myeloid leukemia factor 2	<i>Salmo salar</i>	CAF90637.1	3.00E-68	65	831	1
04-G10	AB595006	myeloperoxidase	<i>Siniperca chuatsi</i>	ABC72122.1	1.00E-121	67	921	1
14-C12	AB595118	NAD(P)H dehydrogenase, quinone 1	<i>Danio rerio</i>	NP_991105.1	6.00E-49	81	591	1
13-C06	AB595103	NADH dehydrogenase 1 alpha subcomplex subunit 13	<i>Salmo salar</i>	ACI69466.1	8.00E-59	84	581	1
04-C10	AB595001	NADH dehydrogenase 1 alpha subcomplex subunit 4-like 2	<i>Osmerus mordax</i>	ACO10104.1	3.00E-37	81	804	1
01-A06	AB594978	NADH dehydrogenase subunit 1	<i>Oplegnathus fasciatus</i>	YP_001974618.1	1.00E-128	86	959	4
06-G12	AB595032	NADH dehydrogenase subunit 2	<i>Oplegnathus fasciatus</i>	YP_001974619.1	4.00E-81	64	911	3
14-D11	AB595120	NADH dehydrogenase subunit 6	<i>Oplegnathus fasciatus</i>	YP_001974629.1	1.00E-54	77	805	5
11-C07	AB595084	NatTECTIN precursor	<i>Salmo salar</i>	ACI67625.1	9.00E-16	68	450	1
14-H08	AB595125	NatTECTIN; Flags: Precursor	<i>Thalassophryne nattereri</i>	Q66S03.1	2.00E-34	59	732	21
01-G11	AB594988	Perforin-1 precursor	<i>Salmo salar</i>	ACI33854.1	8.00E-47	41	860	1
05-F01	AB595014	Peroxiredoxin-1	<i>Anoplopoma fimbria</i>	ACQ58049.1	1.00E-107	96	893	1
13-C08	AB595092	Peroxiredoxin-6	<i>Salmo salar</i>	ACI67008.1	2.00E-58	80	716	1
12-D04	AB595104	Peroxiredoxin-6	<i>Salmo salar</i>	ACI67571.1	1.00E-107	93	808	1
11-G07	AB595086	polycomb group ring finger 6	<i>Danio rerio</i>	NP_001082838.1	3.00E-34	88	638	1
04-B10	AB595000	polyubiquitin	<i>Cricetulus griseus</i>	BAA23488.1	1.00E-134	95	822	1
13-A10	AB595102	prostate stem cell antigen precursor-like	<i>Ictalurus punctatus</i>	ABD85498.1	9.00E-18	52	847	2

Table 2 (Continued)

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
10-D12	AB595078	Proteasome activator complex subunit 1	<i>Oncorhynchus mykiss</i>	ACO08129.1	6.00E-94	81	715	1
02-C10	AB594991	proteasome alpha 1 subunit	<i>Monodelphis domestica</i>	XP_001379009.1	1.00E-137	96	884	1
11-G04	AB595085	Proteasome subunit beta type-4 precursor	<i>Anoplopoma fimbria</i>	ACQ58421.1	7.00E-30	100	703	1
08-D09	AB595054	RAB5A, member RAS oncogene family like	<i>Danio rerio</i>	NP_957264.1	1.00E-105	94	906	1
10-B01	AB595072	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8	<i>Danio rerio</i>	XP_001921350.1	8.00E-32	49	614	1
10-C05	AB595075	ras-related C3 botulinum toxin substrate 2	<i>Pagrus major</i>	AAP20195.1	2.00E-72	98	807	1
10-H10	AB595082	Ras-related protein Rap-1b precursor	<i>Anoplopoma fimbria</i>	ACQ58088.1	2.00E-69	95	876	1
04-F09	AB595005	receptor activity modifying protein 2a	<i>Takifugu obscurus</i>	BAE45306.1	8.00E-63	71	866	1
13-D11	AB595105	receptor for activated protein kinase C s	<i>Oreochromis mossambicu</i>	AAQ91574.1	1.00E-162	97	938	1
10-G01	AB595081	Regulator of G-protein signaling 9-binding protein	<i>Danio rerio</i>	Q504F3.1	4.00E-50	67	800	1
12-E10	AB595097	Renin receptor	<i>Salmo salar</i>	NP_001133563.1	1.00E-105	83	766	1
05-C05	AB595009	ReO_6	<i>Oryzias latipes</i>	BAB83841.1	1.00E-10	58	936	2
14-H10	AB595126	Retinoblastoma-binding protein 6	<i>Salmo salar</i>	ACM08487.1	1.00E-20	100	789	1
08-D01	AB595053	retinol binding protein 7, cellular	<i>Mus musculus</i>	NP_071303.1	3.00E-40	58	888	1
10-C08	AB595076	rhamnose-binding lectin	<i>Channa argus</i>	ACD76075.1	0.084	86	797	1
05-A09	AB595007	Serine protease 27	<i>Salmo salar</i>	NP_001139871.1	2.00E-81	67	861	1
02-A10	AB594990	Serine protease 27 precursor	<i>Esox lucius</i>	ACO14162.1	5.00E-76	67	916	1
03-A06	AB594994	Serine/threonine-protein kinase 17A	<i>Salmo salar</i>	ACI34195.1	1.00E-09	83	862	1
05-E06	AB595013	stress protein HSC70-1	<i>Seriola quinqueradiata</i>	BAG82848.1	1.00E-131	86	866	1
12-B05	AB595089	suppressor of cytokine signaling 3a	<i>Tetraodon nigroviridis</i>	ABC60040.1	6.00E-97	91	852	1
12-H07	AB595099	T-cell receptor beta, type 2	<i>Paralichthys olivaceus</i>	BAB82593.1	1.00E-18	60	735	1
07-C06	AB595037	TGF-beta-inducible nuclear protein 1	<i>Anoplopoma fimbria</i>	ACQ58100.1	1.00E-122	92	842	1
09-F08	AB595064	Thioredoxin	<i>Anoplopoma fimbria</i>	ACQ58191.1	1.00E-38	82	689	1

Table 2 (Continued)

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
01-E12	AB594986	Thymosin beta-12	<i>Lateolabrax japonicus</i>	P33248.2	6.00E-14	100	813	3
13-H01	AB595110	Thymosin beta-a	<i>Esox lucius</i>	ACO13749.1	2.00E-06	59	624	4
07-G01	AB595044	TNF-related apoptosis inducing ligand	<i>Siniperca chuatsi</i>	AAX77404.1	1.00E-38	96	904	1
05-C02	AB595008	translationally-controlled tumor protein	<i>Oryzias latipes</i>	ACO82289.1	7.00E-64	84	886	2
08-E12	AB595055	transmembrane 7 superfamily member 1	<i>Danio rerio</i>	XP_001341229.1	7.00E-86	70	940	1
12-C07	AB595091	Transmembrane protein 128	<i>Salmo salar</i>	NP_001134319.1	6.00E-67	73	802	1
08-F08	AB595057	transmembrane protein 33	<i>Danio rerio</i>	NP_998828.1	1.00E-126	94	936	1
13-H11	AB595113	Transmembrane protein 59 precursor	<i>Salmo salar</i>	ACI33199.1	1.00E-107	73	962	1
01-B03	AB594980	Tubulin alpha-1 chain	<i>Osmerus mordax</i>	ACO09494.1	2.00E-53	99	463	1
12-E06	AB595096	tubulin, alpha 8 like 2	<i>Salmo salar</i>	NP_001133158.1	1.00E-143	98	789	1
04-E03	AB595003	Tumor necrosis factor receptor superfamily member 14 precursor	<i>Osmerus mordax</i>	ACO08877.1	1.00E-55	61	893	3
07-E06	AB595042	type I cytokeratin	<i>Danio rerio</i>	CAK11298.1	2.00E-83	58	898	2
07-D04	AB595039	type I keratin-like protein	<i>Sparus aurata</i>	ACN62548.1	1.00E-120	95	916	2
14-F04	AB595123	type II keratin	<i>Solea senegalensis</i>	BAH56637.1	4.00E-84	97	824	8
08-H08	AB595059	type II keratin E3-like protein	<i>Sparus aurata</i>	AAT44423.1	3.00E-54	98	356	1
04-A04	AB594999	ubiquinol-cytochrome c reductase core I protein	<i>Oncorhynchus mykiss</i>	AF465782_1	1.00E-136	85	870	1
05-C10	AB595010	ubiquitin (ribosomal protein L40)	<i>Schistosoma mansoni</i>	CAZ32225.1	1.00E-151	100	838	2
09-A10	AB595060	ubiquitin	<i>Hydra magnipapillata</i>	XP_002158040.1	3.00E-06	53	774	1
06-C03	AB595022	Ubiquitin	<i>Salmo salar</i>	ACM09817.1	1.00E-130	98	858	1
09-E12	AB595063	Ubiquitin carboxyl-terminal hydrolase 12	<i>Salmo salar</i>	NP_001133405.1	1.00E-158	98	866	1
06-A04	AB595018	Ubiquitin-conjugating enzyme E2 N	<i>Salmo salar</i>	ACM08830.1	0.012	86	535	1
08-A09	AB595051	Ubiquitin-conjugating enzyme E2 variant 3	<i>Salmo salar</i>	NP_001133767.1	1.00E-137	80	938	1
07-H09	AB595048	N-acetylgalactosaminyltransferase 5 (GalNAc-T5)	<i>Danio rerio</i>	XP_001338929.2	1.00E-47	64	894	1
13-G12	AB595109	vertebrate granzyme family	<i>Danio rerio</i>	NP_001108166.1	6.00E-24	72	620	1

Table 2 (Continued)

Clone no.	Accession no.	Putative identification	Closest species	Accession no.	E-value	I ^a	bp	F ^b
10-D04	AB595077	vertebrate tumour differentially expressed protein family	<i>Danio rerio</i>	CAK04623.1	4.00E-53	72	601	1
03-C08	AB594997	virus-induced protein 5	<i>Siniperca chuatsi</i>	AAV65043.1	1.00E-65	83	823	2
07-E02	AB595040	zinc finger protein	<i>Danio rerio</i>	CAQ14162.1	7.00E-22	36	908	1
09-B02	AB595061	Zinc finger protein Gfi-1b	<i>Salmo salar</i>	NP_001133599.1	5.00E-13	42	947	1
07-C04	AB595036	Zinc transporter 4	<i>Salmo salar</i>	NP_001133377.1	5.00E-46	79	731	1
13-F01	AB595107	Zinc transporter SLC39A11	<i>Salmo salar</i>	ACN11096.1	7.00E-43	87	915	1
08-A05	AB595049	Zymogen granule membrane protein 16 precursor	<i>Salmo salar</i>	ACM08996.1	9.00E-30	65	880	3

^a Identity (%).

^b Frequency (time).

The most redundant clones (Table 2) were found in those coding for nattectin. Nattectin induced a significant cellular recruitment into peritoneal cavity of mice, mainly by influx of neutrophils, followed by macrophages, with synthesis of PGE, LTB, IL-1b, IL-6, KC, MCP-1, IL-10, and IL-12 (Tânia *et al.*, 2009).

The second most redundant clones (Table 2) were seen in those coding for beta-2 microglobulin. Beta-2 microglobulin regulates NK cell function by direct contact with NK cell inhibitory receptors (Michaelsson *et al.*, 2001). A role in the anti-tumor immune response (Mori *et al.*, 1999) and viral resistance (Klingel *et al.*, 2003) has also been suggested.

We have sequenced clones for the several types of lectins. The C-type lectin superfamily is comprised of proteins functionally important in glycoprotein metabolism, mechanisms of multi-cellular integration, and immunity (Zelensky and Gready, 2004). Fish mucus contains a variety of antimicrobial substances, such as

lysozyme, proteolytic enzyme, lectin, C-reactive protein and bactericidal peptides (Hjelmeland *et al.*, 1983; Cole *et al.*, 2000; Honda *et al.*, 2000). Because water is an ideal medium for the transmission of bacteria and parasitic microbes, fish are constantly exposed to pathogens via the skin, gills, and alimentary canal. It is generally believed that defense mechanisms against pathogens exist in the gills, and that the gill serves as a mechanical, as well as biochemical, barrier. Indeed, there is evidence that lectins can be found in gill tissue (Mistry *et al.*, 2001; Russell and Lumsden, 2005).

The discovery of novel teleost genes related to the immune response has been accelerated by high-throughput sequencing techniques combined with searches for homologous sequences in public databases. The sequencing of ESTs is especially useful since it simultaneously allows novel gene discovery and gene expression analysis. It also indicates that the range of rock bream gill ESTs identified in this study covers

the known gill functions and therefore should be useful to monitor gill gene expression under different physiological conditions.

In conclusion, this study reports an expressed sequence tag (EST) based gene identification analyzes the gene identification of 1450 ESTs derived from rock bream gill cDNA library. These EST analyses will be useful for the construction of cDNA microarray and recombinant proteins. The application of cDNA microarrays may facilitate research attempting to answer questions concerning immune responses and other protective responses of rock bream upon infection of pathogens.

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