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Appendices

Appendix A

I. *P.monodon* ESTs homologues : Gene expression, regulation and protein synthesis

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh1	40S ribosomal protien S18	Human	P25232	242	1.00E-63	80	94 / 135	1	494
sh3	60S ribosomal protien L9	Drosophila melanogaster	P50882	178	1.00E-58	73	83 / 118	2	584
sh10	60S ribosomal protien L11	Drosophila melanogaster	P46222	225	2.00E-58	82	92 / 131	1	470
sh34	40S ribosomal protien S24	Fugu rubripes	O42387	192	2.00E-48	80	88 / 115	2	602
sh36	16S ribosomal RNA gene, mitochondrial RNA	Penaeus monodon	AF217843	1010	0	91	*/673	-	699
sh66	16S ribosomal RNA gene, mitochondrial RNA	Penaeus monodon	AF105039	278	9.00E-73	83	*/355	-	502
sh102	16S ribosomal RNA gene, mitochondrial RNA	Penaeus monodon	AF105039	632	e-179	96	*/546	-	549
sh195	LMPX of lamprey	Petromyzon marinus	BAA10932	269	2.00E-71	78	86 / 163	1	718
sh212	ribosomal protein S8	Apis mellifera	3396100	256	4.00E-69	69	74 / 190	2	735
sh363	40S ribosomal protein S7	Fugu rubripes	P50894	113	5.00E-25	65	79 / 88	1	299
sh390	QM homolog	Drosophila melanogaster	3123840	159	6.00E-39	77	85 / 100	1	306
sh418	ubiquitin-like protein / ribosomal protein S30	rat	A47416	50	3.00E-12	62	74 / 56	3	369
sh426	elongation factor-1 alpha	Armadillidium	2196950	165	9.00E-41	93	82/160	-3	323
sh436	polyubiquitin	Cricetulus griseus	BAA23488	185	7.00E-47	92	95 / 104	3	315
sh452	60S ribosomal protien L44		P48166	141	1.00E-34	79	90 / 79	-3	326
sh465	ubiquitin-conjugating enzyme E2 - 17 kD	Drosophila melanogaster	P25867	112	1.00E-24	60	68 / 109	2	374
sh601	40S ribosomal protein S32	Drosophila melanogaster	AAF58277	216	2.00E-55	88	90 / 117	-1	473
sh617	elongation factor-1 alpha	Libinia emarginata	AAC03149	313	1.00E-84	93	96 / 160	-3	707
sh621	ribosomal protein S17	Homo sapiens	NP001012	221	4.00E-57	88	94 / 122	-1	503
sh622	26S proteasome regulatory subunit	Anopheles stephens	CAB61220	161	5.00E-65	76	86 / 101	3	760
sh631	eukaryotic translation initiation factor XeIF-4A III	Xenopus laevis	AAB71410	404	1.00E-112	94	97 / 206	-3	810
sh636	40S ribosomal protien S4	Gallus gallus	P47836	306	1.00E-105	72	82 / 193	1	811
sh638	ubiquitin-like protein / ribosomal protein S30	Rattus rattus	A47416	112	2.00E-24	43	62 / 134	2	462
sh642	5S ribosomal protein	Mus musculus	CAA73041	189	4.00E-89	92	97 / 100	1	675

I. *P.monodon* ESTs homologues : Gene expression, regulation and protein synthesis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh657	ubiquitin / ribosomal protein CEP52	<i>Drosophila melanogaster</i>	S23988	249	1.00E-65	91	98 / 128	2	520
sh666	chromatin-specific transcription elongation factor	<i>Homo sapiens</i>	NP009123	201	9.00E-51	41	69 / 215	3	827
sh673	snRNP-associated protein	<i>Danio rerio</i>	AAF72188	115	2.00E-31	58	74 / 95	2	418
sh679	von Hippel-Lindau binding protein 1	<i>Homo sapiens</i>	NP003363	72	3.00E-12	66	84 / 48	3	378
sh690	60S ribosomal protein L17	Rat	P24049	101	5.00E-21	50	65 / 114	1	404
sh697	40S ribosomal protein S24	<i>Fugu rubripes</i>	O42387	198	3.00E-50	74	86 / 126	1	458
sh699	40S ribosomal protien S4	Chiccken	P47836	259	1.00E-105	68	83 / 172	3	812
sh700	60S ribosomal protein -like	<i>Arabidopsiis thaliana</i>	CAB66929	99	1.00E-34	59	72 / 87	2	474
sh701	Elongation factor-1 beta	<i>Bombyx mori</i>	P29522	154	5.00E-37	76	85 / 97	-3	458
sh702	60S ribosomal protein L7A	Chiccken	P32429	240	1.00E-62	56	68 / 232	1	864
sh708	60S ribosomal protein L10A	Rat	P52859	207	2.00E-70	58	71 / 173	2	712
sh711	multicatalytic endopeptidase complex	human	S17521	248	2.00E-82	79	87 / 152	1	760
sh714	elongation factor 2	<i>Drosophila melanogaster</i>	P13060	117	3.00E-42	82	87 / 67	-1	637
sh719	60S ribosomal protein L19	<i>Drosophila melanogaster</i>	P36241	197	6.00E-50	63	74 / 161	2	560
sh730	elongation factor -1 alpha	<i>Libinia emarginata</i>	AAC03149	293	2.00E-78	88	92 / 160	-1	718
sh736	ribosomal protein L37	Human	NP000988	90	4.00E-24	64	74 / 57	-1	335
sh738	60S ribosomal protein L17	Rat	P24049	147	2.00E-58	67	79 / 110	-3	620
sh746	ribosomal protein S3	Rat	R3RT3	253	8.00E-67	83	86 / 151	-2	522
sh753	60S ribosomal protein L14	<i>Rattus norvegicus</i>	Q63507	129	2.00E-29	44	61 / 145	-3	644
sh765	protein complex subunit 34	<i>Homo sapiens</i>	NP005722	97	2.00E-19	79	84 / 58	-1	738
sh769	proteasome subunit beta type 2	<i>Homo sapiens</i>	NP002785	209	3.00E-53	52	69 / 195	1	764
sh780	transcription initiation factor TFIID 110kD subunit	<i>Drosophila melanogaster</i>	P47825	104	1.00E-21	42	54 / 148	3	955
sh796	cellular nucleic acid binding protein	<i>Xenopus laevis</i>	AAA81168	137	1.00E-36	43	52 / 161	1	751
sh811	ribosomal protein L30	Rat	NP000980	154	1.00E-42	75	87 / 97	-1	456

I. *P.monodon* ESTs homologues : Gene expression, regulation and protein synthesis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh813	probable 60S ribosomal protein L17	Caenorhabditis elegans	O01802	101	4.00E-46	55	74 / 76	2	750
sh815	33 kDa transcription co-activator	Homo sapiens	NP004261	213	1.00E-54	60	78 / 162	2	845
sh821	ribosomal protein S16	Human	NP001011	139	6.00E-33	87	96 / 74	-2	272
sh831	proteasome beta chain precursor	Mus musculus	P99026	65	9.00E-21	43	65 / 66	-2	673
sh840	probable threonyl-tRNA synthetase	Caenorhabditis elegans	P52709	377	1.00E-112	71	79 / 245	2	826
sh858	protein arginine methyltransferase	Mus musculus	AAD41265	166	3.00E-40	44	52 / 243	2	689
sh864	probable reverse transcriptase	house mosquito	B56679	49	3.00E-05	32	46 / 137	-2	454
sh871	putative large subunit ribosomal protein rpL 44	Aedes triseriatus	AAF87576	167	5.00E-41	74	81 / 104	1	430
sh890	RpS25 gene product	Drosophila melanogaster	AAF54605	124	4.00E-28	75	89 / 78	1	397
sh891	eukaryotic translation initiation factor XelF-4A III	Xenopus laevis	AAB71410	458	1.00E-133	92	97 / 240	1	815
sh905	RpS 9 gene product	Drosophila melanogaster	AAF50249	227	9.00E-78	81	87 / 142	2	626
sh925	ribosomal protein S8	Apis mellifera	AAC28863	291	6.00E-78	73	77 / 200	2	674
sh932	brain protein I3	Homo sapiens	NP056194	78	1.00E-13	57	71 / 54	1	832
sh941	TRNKYRASE	Homo sapiens	NP003738	67	8.00E-11	33	44 / 144	1	436
sh943	60S ribosomal protein L5	Bombyx mori	O76190	228	4.00E-59	62	78 / 170	2	625
sh952	ribosomal protein S29	human	NP001023	69	7.00E-12	69	75 / 46	-1	210
sh959	5S ribosomal protein	Mus musculus	CAA73041	237	2.00E-93	95	98 / 125	-2	689
sh961	40S ribosomal protein S15A	Drosophila melanogaster	P48149	238	2.00E-62	89	96 / 124	1	409
sh968	ribosomal protein S8	Apis mellifera	AAC28863	291	6.00E-78	73	77 / 200	2	675
sh974	ribosomal protein S27a	Human	NP002945	212	1.00E-54	73	75 / 146	2	465
sh979	brain protein I3	Homo sapiens	NP056194	78	9.00E-14	57	71 / 54	3	723
sh993	eukaryotic translation initiation factor 3, subunit 2	Mus musculus	NP061269	164	1.00E-39	74	88 / 98	-3	704
sh998	protein synthesis initiation factor 4A	Mus musculus	CAA40268	199	2.00E-84	79	84 / 124	2	657
sh1012	RpS25 gene product	Drosophila melanogaster	AAF54605	128	8.00E-29	76	89 / 80	-1	960

I. *P.monodon* ESTs homologues : Gene expression, regulation and protein synthesis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh1014	ribosomal protein S27a	Human	NP002945	193	1.00E-48	70	73 / 136	1	443
sh1031	double stranded RNA binding nuclear protein, ILF3	Homo sapiens	CAC01407	53	2.00E-06	37	58 / 83	1	535
sh1034	ribosomal protein S26	Human	NP037356	180	8.00E-45	82	90 / 101	-3	579
sh1040	proteasome subunit alpha type 6	Mus musculus	NP036098	133	2.00E-30	66	80 / 98	-3	816
sh1044	ribosomal protein S30	Rattus rattus	A47416	112	5.00E-24	43	62 / 134	1	751
sh1084	ribosomal protein L26	Homo sapiens	AAA60279	200	8.00E-51	73	83 / 134	2	460
sh2016	elongation factor-1 alpha	Libinia emarginata	AAC03149	326	2.00E-88	93	96 / 161	-1	684
sh2011	elongation factor-1 alpha	Salmo salar	AAG38613	404	E-111	83	90 / 224	1	769
sh2028	40S ribosomal protein S3A	Oryzias latipes	O73813	152	1.00E-36	62	83 / 113	-1	348
sh2030	ubiquitin - conjugating enzyme E2-24 kDa	Drosophila melanogaster	P52485	276	3.00E-73	99	99 / 126	3	862
sh2032	ribosomal protein S3a	Ophiophagus hannah	AAK09383	225	3.00E-80	66	80 / 158	1	738
sh2036	60S ribosomal protein L6	Homo sapiens	XP_016700	118	6.00E-26	48	67 / 130	1	707
sh2043	nascent polypeptide-associated complex alpha chain	Mus musculus	T30827	91	1.00E-17	46	52 / 115	-2	619
sh2051	chromatin-specific transcription elongation factor	Homo sapiens	NP_009123	132	3.00E-30	44	67 / 146	1	546
sh2063	40S ribosomal protein S3a	Oryzias latipes	O73813	344	1.00E-93	67	84 / 238	3	774
sh2033	putative reverse transcriptase	<i>Takifugu rubripes</i>	AAD47076	101	8.00E-21	38	56/164	-1	504

II. *P.monodon* ESTs homologues : Internal / external structure and motility

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh14	actin, clone 403	shrimp	P18603	247	4.00E-65	89	89 / 133	-2	531
sh30	actin	Atlantic horseshoe crab	P41339	344	e-125	98	98 / 167	3	800
sh58	profilin	Drosophila melanogaster	P25843	99	3.00E-20	41	59 / 120	3	800
sh171	actin , cytoskeletal 1	Lytechinus pictus	P53465	240	8.00E-73	95	95 / 124	1	716
sh184	beta actin	Morone saxatilis	567192	136	5.00E-42	98	98 / 66	2	302
sh213	Actin , alpha cardiac muscle	African clawed frog	P04751	228	6.00E-67	85	87 / 131	2	800
sh513	tubulin alpha-2 / alpha-4 chain	Patella vulgata	P41383	68	1.00E-11	93	96 / 30	2	159
sh602	tubulin beta	Homo sapiens	O808321A	233	1.00E-107	69	72 / 164	3	885
sh626	stromelysin-3 precursor	Xenopus laevis	Q11005	96	3.00E-25	37	4949/83	2	661
sh627	cytoplasmic beta chain	Xenopus laevis	AAC27796	201	2.00E-87	97	98 / 99	2	711
sh637	tubulin beta-1 chain	Homarus americanus	Q25009	302	3.00E-81	96	98 / 145	1	888
sh656	actin 2	carrot	P23344	309	1.00E-58	92	96 / 63	-2	731
sh658	alpha-2-tubulin	Gecarcinus lateralis	AAC47523	118	4.00E-29	65	66 / 93	-3	542
sh664	tubulin beta-1 chain	Homarus americanus	Q25009	187	4.00E-99	80	81 / 111	1	676
sh674	actin 1	Dugesia polychroa	AAC38981	95	3.00E-28	93	99 / 46	2	444
sh693	actin, clone 403	Artemia sp	P18603	302	1.00E-98	79	80 / 198	1	815
sh733	profilin	Drosophila melanogaster	P25843	64	1.00E-13	36	57 / 91	-3	819
sh750	beta tub 56D gene product (alt 1)	Drosophila melanogaster	AAF57556	369	1.00E-141	94	97 / 189	1	938
sh791	beta actin	Cricetinae gen. Sp.	AAB66487	319	2.00E-86	77	78 / 221	1	698
sh799	actin	Setaria digitata	AAD13153	183	1.00E-72	76	77 / 121	3	786
sh819	calponin homolog	Schistosoma japonicus	AAD11976	111	2.00E-27	47	63 / 133	3	615
sh839	ubiquitin-like protein SMT3	Caenorhabditis elegans	P55853	136	4.00E-31	74	83 / 87	3	849
sh852	actin, clone 403	Artemia sp	P18603	101	5.00E-44	100	100 / 46	1	288
sh859	actin	Lymantria dispar	AAD54427	174	4.00E-46	100	100 / 84	3	438

II. *P.monodon* ESTs homologues : Internal / external structure and motility (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh860	actin, clone 403	brain shrimp	P18603	219	3.00E-64	94	94 / 112	-3	560
sh866	dynein light chain, cytoplasmic	<i>Drosophila melanogaster</i>	Q24117	182	3.00E-45	95	99 / 88	3	729
sh872	Tubulin alpha-3 chain	<i>Homarus americanus</i>	Q94572	284	3.00E-76	90	91 / 155	-1	522
sh873	beta actin	<i>Rivulus marmoratus</i>	AAF26678	177	6.00E-44	86	80/174	2	466
sh930	myosin regulatory light chain	<i>Drosophila melanogaster</i>	P40423	143	3.00E-33	81	89 / 80	1	911
sh933	cytoplasmic beta actin	<i>Xenopus laevis</i>	AAC27769	443	1.00E-126	95	95 / 231	-3	907
sh940	actin 3	<i>Limulus polyphemus</i>	P41340	347	1.00E-109	98	98 / 168	2	671
sh946	actin, clone 403	<i>Artemia</i> sp	P18603	414	1.00E-120	98	99 / 207	-2	784
sh984	actin	<i>Strongylocentrotus purpuratus</i>	AAC41544	57	2.00E-08	100	100 / 24	-3	281
sh986	tubulin alpha-1 chain	<i>Homarus americanus</i>	Q25008	409	1.00E-171	88	92 / 219	2	985
sh 1007	ferritin 2 light chain homolog	<i>Drosophila melanogaster</i>	AAF07876	47	2.00E-04	25	49/167	2	904
sh1024	tubulin alpha-1 chain	<i>Homarus americanus</i>	Q25008	362	1.00E-102	97	98 / 175	-1	826
sh1036	actin, clone 403	brain shrimp	P18603	243	1.00E-127	97	98 / 121	1	794
sh1042	SMC 1 protein	<i>Drosophila melanogaster</i>	CAB76376	317	1.00E-85	62	76 / 256	1	930
sh1070	actin related protein	<i>Lumbricus rubellus</i>	CAA11175	108	7.00E-23	92	99 / 53	2	677
sh2007	actin, cytoplasmic A3	<i>Bombyx mori</i>	P04829	369	E-110	98	98 / 173	3	642
sh2012	actin, cytoplasmic A3	<i>Bombyx mori</i>	P04829	337	6.00E-93	95	95 / 165	2	569
sh2020	beta - actin	<i>Canis familiaris</i>	AAB71610	315	3.00E-85	86	89 / 174	-2	676
sh2024	beta - actin	<i>Penaeus vannamei</i>	AAG16253	473	E-133	99	99 / 223	2	796
sh2066	beta - actin	<i>Penaeus vannamei</i>	AAG16253	553	E-156	96	96 / 282	2	909
sh2067	beta - actin	<i>Penaeus vannamei</i>	AAG16253	223	E-150	99	99 / 103	2	891

III. *P.monodon* ESTs homologues : Metabolism

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh31	cytochrome c oxidase subunit I	<i>Penaeus notialis</i>	CAB40364	478	e-134	91	96 / 263	-1	800
sh39	cytochrome B	fruit fly	P07704	380	e-105	67	74 / 278	3	867
sh75	cytochrome B	fruit fly	P07704	269	1.00E-72	62	72 / 209	-2	680
sh192	guanine nucleotide-binding protein G	migratory locust	P38404	220	8.00E-57	95	96 / 110	2	727
sh393	pterin-4a-carbinone dehydratase	<i>Drosophila melanogaster</i>	3283431	92	1.00E-18	65	85 / 60	2	280
sh408	cytochrome c oxidase subunit I	<i>Penaeus notialis</i>	CAB40364	53	4.00E-07	85	88 / 28	-3	87
sh411	bovine NADH-ubiquinone oxidoreductase	<i>Caenorhabditis elegans</i>	CAB01876	78	2.00E-14	41	60 / 86	2	288
sh446	ATPase subunit 6	<i>Penaeus notialis</i>	CAB40367	59	9.00E-09	72	88 / 36	2	245
sh447	cytochrome c oxidase subunit III	<i>Penaeus notialis</i>	CAB40368	83	3.00E-16	79	87 / 48	1	144
sh604	cytochrome c oxidase subunit II	<i>Penaeus monodon</i>	AAF43375	227	1.00E-58	75	79 / 160	3	895
sh605	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	487	1.00E-137	92	96 / 267	-3	817
sh612	cytochrome c oxidase subunit III	<i>Penaeus monodon</i>	AAF43376	370	1.00E-105	82	84 / 231	-2	765
sh613	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	253	1.00E-66	74	80 / 179	1	544
sh632	peroxisomal Ca-dependent solute carrier	<i>Oryctolagus cuniculus</i>	AAB69156	242	4.00E-63	59	75 / 198	-2	942
sh647	cytochrome c oxidase subunit Via precursor	<i>Thunnus niloticus</i>	AAF04404	70	2.00E-18	48	76 / 56	-3	503
sh650	guanine nucleotide-binding protein beta subunit	<i>Oreochromis niloticus</i>	O42249	198	3.00E-82	78	88 / 114	3	747
sh653	GTP-binding nuclear protein RNA	<i>Drosophila melanogaster</i>	AAF30287	85	4.00E-16	60	70 / 74	-1	608
sh691	nucleoside diphosphate kinase	<i>Columba livia</i>	Q90380	194	4.00E-49	73	84 / 126	3	456
sh695	non-selenium glutathione phospholipid hydroperoxidase peroxidase	<i>Sus scrofa</i>	CAB65456	129	1.00E-29	79	57 / 78	-2	872
sh698	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	413	1.00E-115	91	95 / 225	-3	691
sh703	aldehyde reductase	<i>Sus scrofa</i>	A59021	98	6.00E-37	52	66 / 96	-2	708
sh704	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	281	1.00E-101	68	73 / 218	1	836
sh707	ATP synthase subunit C	<i>Manduca sexta</i>	AAF16705	49	6.00E-11	70	79 / 31	-3	829

III. *P.monodon* ESTs homologues : Metabolism (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh712	ATP synthase subunit C	Manduca sexta	AAF16705	71	4.00E-12	58	61 / 78	-3	777
sh716	vacuolar ATP synthase subunit G	Manduca sexta	Q25532	100	2.00E-20	79	68 / 77	2	626
sh721	NADH dehydrogenase subunit 4	Penaeus monodon	AAF43381	213	2.00E-54	60	64 / 185	1	769
sh729	cytochrome c oxidase subunit III	Penaeus monodon	AAF43376	400	1.00E-110	80	83 / 255	3	781
sh731	PKCq-interacting protein PICOT	Homo sapiens	AAF28841	174	3.00E-52	48	67 / 185	3	707
sh734	NADH dehydrogenase subunit II	Penaeus monodon	AAF43379	365	1.00E-100	71	77 / 266	2	804
sh739	ATP synthase FO subunit 6	Penaeus monodon	AAF43372	300	1.00E-80	70	75 / 220	-3	795
sh740	cytochrome c oxidase subunit II	Penaeus monodon	AAF43375	326	7.00E-93	81	83 / 206	1	696
sh756	ATP synthase FO subunit 6	Penaeus monodon	AAF43372	229	1.00E-72	84	90 / 132	2	749
sh758	cytochrome c oxidase subunit I	Penaeus monodon	AAF43374	337	1.00E-98	92	96 / 182	1	673
sh766	protein-glutamine	Epidermal	P22758	60	3.00E-08	33	53 / 86	1	750
sh763	cytochrome c oxidase subunit I	Penaeus notialis	CAB40364	289	2.00E-77	66	73 / 232	2	700
sh792	ATP synthase FO subunit 6	Penaeus monodon	AAF43372	229	1.00E-72	84	90 / 132	2	817
sh793	COP9 (constitutive photomorphogenic) subunit 4	Mus musculus	NP036131	217	5.00E-78	81	87 / 133	-2	829
sh809	cytochrome c oxidase subunit II	Penaeus monodon	AAF43375	336	1.00E-91	80	83 / 220	1	792
sh816	chain A, Triosephosphate isomerase	chicken	1TPW	169	3.00E-54	54	70 / 148	2	654
sh828	chain H, cytochrome Bc1 complex	chicken	1BCC	79	4.00E-14	49	66 / 67	2	593
sh830	RAS-related protein RAB-1A	Lymnaea stagnalis	Q05974	351	8.00E-96	88	90 / 198	3	923
sh844	beta2-chimeric	Rat	B53764	152	2.00E-38	45	63 / 165	1	799
sh849	glucose- β -phosphatase	Mus musculus	CAA87708	143	2.00E-33	38	58 / 185	3	768
sh861	ATP synthase FO subunit 6	Penaeus monodon	AAF43372	177	5.00E-44	65	70 / 140	-2	456
sh883	cytochrome c oxidase subunit I	Penaeus monodon	AAF43374	378	1.00E-104	72	78 / 271	3	815
sh884	GTP-binding nuclear protein RHEB homolog	Drosophila melanogaster	Q9VDN8	265	4.00E-70	69	83 / 182	2	726
sh896	calmodulin	Drosophila melanogaster	P07181	52	5.00E-06	100	100 / 24	1	714

III. *P.monodon* ESTs homologues : Metabolism (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh900	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	466	1.00E-130	92	96 / 250	1	750
sh916	acyl coenzyme A dehydrogenase, long chain	<i>Rattus norvegicus</i>	NP036951	304	1.00E-81	73	86 / 196	3	930
sh924	cytochrome c oxidase subunit III	<i>Penaeus monodon</i>	AAF43376	211	2.00E-98	94	95 / 109	-2	813
sh927	NADH dehydrogenase subunit 5	<i>Penaeus monodon</i>	AAF43383	391	1.00E-108	79	88 / 241	-2	898
sh937	calmodulin	<i>Drosophila melanogaster</i>	P07181	299	2.00E-80	100	100 / 149	-1	747
sh969	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	431	1.00E-120	74	79 / 301	3	906
sh976	ATP synthase FO subunit 6	<i>Penaeus monodon</i>	AAF43372	293	3.00E-85	84	91 / 169	2	823
sh990	cytochrome c oxidase subunit III	<i>Penaeus monodon</i>	AAF43376	396	1.00E-109	80	82 / 257	-3	794
sh996	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	214	9.00E-55	57	63 / 239	-2	725
sh999	cytochrome c oxidase subunit VIIc	Mouse	NP031775	61	5.00E-09	55	72 / 52	2	417
sh1004	putative steroid dehydrogenase	Mouse	NP062631	148	8.00E-35	40	64 / 178	2	728
sh1009	ATPsyn-gamma gene product	<i>Drosophila melanogaster</i>	AAF56932	262	3.00E-69	54	71 / 241	2	710
sh1072	glucose-6-phosphatase	<i>Mus musculus</i>	CAA87708	143	2.00E-33	38	58 / 185	3	721
sh2002	NADH-ubiquinone oxidoreductase 42 kDa subunit	<i>Drosophila melanogaster</i>	P91929	155	6.00E-37	55	75 / 125	-1	710
sh2015	cytochrome c oxidase subunit I	<i>Penaeus notialis</i>	CAB40364	295	5.00E-79	67	72 / 228	3	683
sh2050	cytochrome c oxidase subunit III	<i>Penaeus notialis</i>	CAB40368	206	1.00E-52	78	81 / 128	-1	523
sh2053	ATPase subunit 6	<i>Peaneus notialis</i>	CAB40367	195	3.00E-72	62	70 / 156	-2	748
sh2056	ATP synthase alpha chain, mitochondrial precursor	<i>Drosophila melanogaster</i>	P35381	81	2.00E-14	66	80 / 57	-2	630
sh1029	stromal cell derived factor 2	<i>Mus musculus</i>	NP_033169	163	2.00E-39	46	59 / 158	3	800
sh1065	Apg12	<i>Homo sapiens</i>	NP_004698	139	2.00E-32	59	72 / 111	3	724

IV. *P.monodon* ESTs homologues : Defence and homeostasis

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives % / length aa	Frame	Sequence length (bp)
sh13	antilipoplysaccharide factor	Atlantic horseshoe crab	A23931	86	2.00E-16	37	57 / 95	2	486
sh20	antilipoplysaccharide factor	Atlantic horseshoe crab	A23931	86	2.00E-16	37	57 / 95	2	486
sh37	thymosin beta-11	bovin	CAA21832	122	4.00E-27	53	61 / 128	2	800
sh49	hemocyte protein-glutamine gamma-glutamyltransferase	Tachypleus tridentatus	Q05187	60	2.00E-08	27	51 / 104	3	700
sh71	antilipoplysaccharide factor	Atlantic horseshoe crab	A23931	86	2.00E-16	37	57 / 95	2	500
sh64	heat-shock cognate 70 kD	Trichoplusia ni	1495233	150	3.00E-36	94	95 / 76	1	298
sh67	hemocyte protease-1	Manduca sexta	AAB94557	74	1.00E-12	42	57 / 102	3	700
sh189	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	85	1.00E-16	52	65 / 72	1	352
sh415	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	156	5.00E-11	52	65 / 72	1	789
sh448	protein-glutamine gamma-glutamyltransferase	Tachypleus tridentatus	A45321	148	2.00E-35	58	76 / 117	2	387
sh449	serine proteinase 14 A	Anopheles gambiae	AAD38334	38	7.00E-03	51	76 / 27	2	422
sh467	pro-phenoloxidase activating enzyme-I precursor	Holotrichia diomphalia	BAA34642	51	3.00E-06	46	64 / 49	1	340
sh606	P lysozyme structural	Mouse	NPO38618	97	2.00E-19	42	60 / 129	1	684
sh607	peptidyl-prolyl cis-trans isomerase 5	Caenorhabditis elegans	P52013	222	5.00E-60	68	75 / 164	1	861
sh609	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	64	2.00E-09	41	46 / 80	-1	579
sh610	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	125	5.00E-28	40	56 / 172	2	860
sh644	heat shock protein 90	chicken	HHCH90	115	9.00E-27	64	70 / 96	-3	618
sh655	90 kDa heat shock protein HSP83	Spodoptera frugiperda	AAG44630	78	2.00E-14	50	56 / 117	-3	660
sh665	penaeidin-3c precursor	Penaeus vannamei	P81060	52	2.00E-06	39	48 / 64	1	654
sh685	prophenoloxidase	Penaeus monodon	AAD45201	202	1.00E-51	79	80 / 130	3	465
sh722	peptidyl-prolyl cis-trans isomerase	Blattella germani	P54985	170	4.00E-53	85	91 / 94	3	741
sh742	peptidyl-prolyl cis-trans isomerase 5	Caenorhabditis elegans	P52013	248	5.00E-65	68	74 / 186	1	877
sh737	Penaeidin -3c precursor	Penaeus vannamei	P81060	64	1.00E-09	84	90 / 33	3	666
sh744	Penaeidin -3c precursor	Penaeus vannamei	P81060	68	6.00E-11	85	90 / 35	2	610

IV. *P.monodon* ESTs homologues : Defence and homeostasis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives % / length aa	Frame	Sequence length (bp)
sh771	protein-kinase c inhibitor	bovine	A35350	160	2.00E-38	67	80/115	1	756
sh774	Penaeidin -3c precursor	Penaeus vannamei	P81060	64	9.00E-10	84	90 / 33	3	601
sh776	Proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	135	5.00E-31	43	53 / 166	1	653
sh777	clottable protein	Penaeus monodon	AF089867	472	E-123	98	* / 265	-	434
sh827	clottable protein	Penaeus monodon	AF089867	472	E-130	98	* / 278	-	908
sh834	Penaeidin -3c precursor	Penaeus vannamei	P81060	64	2.00E-15	84	90 / 33	1	645
sh835	Thymosin beta-9 and beta-8	Bovin	P21752	47	2.00E-04	68	77 / 32	-1	776
sh857	probable cathepsin B-like cycteine proteinase,29K precursor	Sarcophaga peregrina	S38939	73	6.00E-18	81	88 / 38	-3	659
sh862	protein-glutamine gamma-glutamyltransferase	Tachypleus tridentatus	A45321	81	9.00E-15	35	59/111	3	719
sh869	P lysozyme structural	Mouse	NPO38618	103	1.00E-21	53	66 / 97	-1	530
sh876	protinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	61	8.00E-11	51	65 / 49	2	584
sh882	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	48	5.00E-05	44	50 / 47	1	651
sh889	whey acidic protein	Macropus eugenii	CAB90357	50	3.00E-05	49	35 / 76	3	682
sh912	anti-lipoplysaccharide factor	Atlantic horseshoe crab	A23931	84	9.00E-16	40	61 / 95	2	541
sh913	chaperonin containing t-complex polypeptide 1	Homo sapiens	AAC96010	247	9.00E-67	65	84/183	1	836
sh914	hepatic lectin	Gallus gallus	P02707	167	3.00E-11	30	50 / 113	2	572
sh918	thymosin beta-11	Oncorhynchus mykiss	CAA21832	90	1.00E-17	52	64 / 90	2	872
sh955	T-complex protein 1, Zeta subunit	Oryctolagus cuniculus	O77622	129	7.00E-40	59	77 / 106	-3	637
sh960	thymosin beta-11	Oncorhynchus mykiss	CAA21832	81	1.00E-14	48	60 / 87	-1	876
sh970	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	66	2.00E-10	40	45 / 79	2	683
sh971	clottable protein	Penaeus monodon	AF089867	472	E-133	98	* / 278	-	455
sh972	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	67	1.00E-10	40	45 / 97	-1	495
sh1018	Penaeidin-2 precursor	Penaeus vannamei	P81057	50	1.00E-51	50	57 / 52	3	465
sh1026	Catalase	Campylobacter jejuni	Q59296	62	3.00E-09	93	93 / 29	1	575

IV. *P.monodon* ESTs homologues : Defence and homeostasis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives % / length aa	Frame	Sequence length (bp)
sh1028	Thymosin beta-9 and beta-8	bovin	P21752	46	2.00E-04	68	77 / 32	-3	730
sh1046	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	68	5.00E-11	50	58 / 67	1	477
sh1064	protein C	Mouse	NP032960	66	3.00E-10	41	63 / 75	2	708
sh1069	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	133	3.00E-30	41	50 / 187	1	811
sh2003	hemocyte protein-glutamine gamma-glutamyltransferase	Tachypleus tridentatus	Q05187	193	3.00E-48	44	61 / 225	2	746
sh2005	cyclophilin 18	Oryctolagus cuniculus	AAF22215	225	8.00E-58	66	72 / 162	-2	756
sh2010	heat-shock protein 70 kDa	Hydra magnipapillata	Q05944	220	2.00E-56	77	90 / 126	3	806
sh2014	Putative chaperonin containing t-complex polypeptide	Ochlerotatus triseriatus	AAF87577	318	5.00E-86	73	89 / 213	1	728
sh2035	perlucin	Haliotis laevigata	S78774	86	7.00E-16	36	54 / 128	-3	575
sh2037	heat shock protein 10	Gallus gallus	AAB86581	116	2.00E-25	58	73 / 99	3	665
sh2039	peptidyl-prolyl cis-trans isomerase 5	Drosophila melanogaster	P25007	145	3.00E-68	70	81 / 92	2	816
sh2045	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	38	1.00E-07	45	66 / 33	-1	860
sh2061	anti-lipopolysaccharide factor	Atlantic horseshoe crab	A23931	86	4.00E-16	43	65 / 81	1	647
sh2062	crustin	Carcinus maenas	CAB51030	51	2.00E-05	35	42 / 70	3	703
sh2069	Penaeidin - 3c precursor	Penaeus vannamei	P81060	46	6.00E-04	73	76 / 26	1	779
sh194	glutathione peroxidase	human	A45207	91	3.00E-18	62	76 / 69	1	331
sh1021	peroxidase	Aedes aegypti	AAC97504	104	1.00E-21	37	52/98	2	747

V *P.monodon* ESTs homologues : Signalling and communication

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh641	Shak-8 (lethal) protein	Drosophila melanogaster	AAB34769	66	5.00E-17	35	65 / 68	-2	788
sh671	agrin precursor	Homo sapiens	AAC39776	43	6.00E-06	36	48 / 63	3	428
sh749	asialoglycoprotein receptor	Mus musculus	2022211A	50	2.00E-05		45		922
sh790	11-1 polypeptide	Plasmodium faciparum	CAA30336	44	6.00E-04	30	56 / 69	2	565
sh837	low-density lipoprotein receptor relate protein - deleted in tumor	Homo sapiens	NP061027	74	1.00E-12	38	51 / 93	2	751
sh874	translocon-associated protein	Canis familiaris	P23438	160	8.00E-39	54	73 / 146	1	499
sh881	kupffer cell receptor	Rattus norvegicus	P10716	77	1.00E-13	28	44 / 179	1	670
sh902	sex-lethal protein homolog	Ceratitis capitata	O61374	128	1.00E-50	57	74 / 101	2	615
sh907	LIV-1 protein, estrogen regulated	Homo sapiens	NP036451	48	1.00E-04	44	67 / 47	3	934
sh2052	moesin/ezrin/radixin	Drosophila melanogaster	AAA19857	184	2.00E-48	62	72 / 150	3	887

VI. *P.monodon* ESTs homologues : Cell division / DNA synthesis, repair and replication

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh634	H3	xenopus laevis	CAA26808	72	8.00E-12	100	100 / 34	-3	800
sh922	SPARC-related protein	Mus musculus	AAD41590	50	3.00E-05	45	46/51	3	657
sh949	histone 1	Mytilus edulis	CCA11816	75	9.00E-13	65	72 / 63	-2	750
sh962	histone 1	Mytilus edulis	CCA11812	96	3.00E-19	65	75 / 76	-1	678
sh963	H3 histone	Homo sapiens	NP002098	165	5.00E-40	100	100/54	-3	927

VII. *P.monodon* ESTs homologues : Non identified function

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh429	15 kDa selenoprotein	Homo sapiens	NP004252	80	8.00E-15	49	65 / 95	2	332
sh35	Hypothetical 68.8kD protein	Yeast	P53863	45	5.00E-04	44	65 / 38	3	800
sh129	Hypothetical 39.6kD protein	Yeast	P36160	98	4.00E-20	47	60 / 120	-2	576
sh182	Hypothetical 68.8kD protein	Yeast	P53863	45	5.00E-04	44	65 / 38	1	775
sh615	hypothetical 36.9 kDa protein	Caenorhabditis elegans	AAF54945	44	5.00E-10	46	63 / 47	1	515
sh677	KIAA1177 protein	Homo sapiens	BAA86491	102	2.00E-21	66	78 / 74	3	408
sh743	hypothetical protein	Arabidopsis thaliana	AAF04190	175	4.00E-43	65	77 / 125	2	920
sh762	hypothetical protein F 26E4.9	Caenorhabditis elegans	T21416	96	3.00E-19	47	69 / 90	2	564
sh843	hypothetical protein 18K protein	goldfish-mitochondrion	JC1348	41	1.00E-04	55	65/95	1	920
sh868	hypothetical protein R17.2	Caenorhabditis elegans	T24234	76	3.00E-13	50	55/95	1	636
sh1016	KIAA0924 protein	Homo sapiens	NP055712	57	2.00E-07	45	64 / 46	2	666
sh1027	KIAA0152 gene product	Homo sapiens	NP055545	173	1.00E-42	58	72 / 149	2	531
sh983	KIAA0670 protein	Homo sapiens	NP055792	115	7.00E-25	41	47 / 295	1	737
sh2034	selenoprotein w muscle 1	Rattus norvegicus	NP_037159	111	2.00E-23	62	74 / 82	1	829
sh2068	hypothetical protein	Drosophila melanogaster	S15691	174	3.00E-42	44	58 / 214	-2	872
sh1010	selenoprotein w muscle 1	Rattus norvegicus	NP037159	108	8.00E-23	62	74 / 82	1	614
sh997	Ha3611 gene product	Homo sapiens	BAA07646	59	2.00E-08	62	87/40	2	371
sh2020	CG6866 gene product	Drosophila melanogaster	AAF53295	73	7.00E-18	36	49/140	1	710
sh2071	po 1 polyprotein	Volvox carteri	S32437	46	5.00E-04	62	84/27	2	573
sh880	tPhLP	Mus musculus	AF146793	161	7.00E-39	41	55/208	1	834
sh845	Probeta 2 gene product	Drosophila melanogaster	AAF49685	240	2.00E-62	66	78/181	-2	724
sh1032	CG14429 gene product	Drosophila melanogaster	AAF46232	93	3.00E-18	66	82/59	1	767

VII. *P.monodon* ESTs homologues : Non identified function

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
sh901	Mo23 gene product	<i>Drosophila melanogaster</i>	AAF4932	101	1.00E-20	88	95/53	1	941
sh786	hypothetical protein F08F1.8	<i>Caenorhabditis elegans</i>	T32473	75	1.00E-12	37	52/99	-1	898
sh787	hypothetical protein Y45F10D.4	<i>Caenorhabditis elegans</i>	T26931	224	7.00E-58	80	91/133	3	875
sh1073	hypothetical protein (Y 14016)	<i>Escherichia coli</i>	CAA74339	67	1.00E-10	76	76/43	-2	707
sh817	hrp 65-3 isoform	<i>Chironomus tentans</i>	CAC42830	52	8.00E-06	25	45/137	1	843
sh920	selenoprotein w muscle 1	<i>Rattus norvegicus</i>	NP037159	108	7.00E-23	62	51/82	2	608
sh786	hypothetical protein F08F1.8	<i>Caenorhabditis elegans</i>	T32473	75	1.00E-12	37	52 / 99	-1	898
sh787	hypothetical protein Y45F10D.4	<i>Caenorhabditis elegans</i>	T26931	224	7.00E-58	80	91 / 133	3	875
sh1073	hypothetical protein (Y 14016)	<i>Escherichia coli</i>	CAA74339	67	1.00E-10	76	76 / 43	-2	707
sh901	Mo25 gene product	<i>Drosophila melanogaster</i>	AAF49432	101	1.00E-20	88	95 / 53	1	941
sh920	selenoprotein w muscle 1	<i>Rattus norvegicus</i>	NP037159	108	7.00E-23	62	74 / 82	2	608

VIII. *V. harveyi*-infected *P. monodon* ESTs homologues gene expression and protein synthesis

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF3	ribosomal protein L28	Homo sapiens	NP_000982	76	1.00E-13	47	64 / 73	2	362
IF11	ribosomal protein L31	Heliothis virescens	CAC19413	138	3.00E-32	55	67 / 124	1	395
IF23	60s ribosomal protein L13	Arabidopsis thaliana	BAA92738	149	1.00E-39	59	72 / 115	1	514
IF24	40s ribosomal protein S4	Gallus gallus	P47836	251	3.00E-66	71	81 / 156	3	481
IF26	60s ribosomal protein L8	Aedes albopictus	P41569	245	3.00E-70	66	80 / 163	2	573
IF31	Proteasome 25 kDa subunit	Drosophila melanogaster	P40301	270	6.00E-72	72	79 / 173	1	520
IF43	60s ribosomal protein L3	Toxocara canis	P49149	271	5.00E-72	59	71 / 215	3	676
IF65	ribosomal protein S15	Homo sapiens	NP_001009	144	4.00E-34	52	57 / 144	2	480
IF69	ribosomal protein S17	Homo sapiens	NP_001012	228	3.00E-59	88	94 / 122	3	450
IF79	60a ribosomal protein L27A	Rattus rattus	P18445	196	1.00E-49	65	75 / 148	2	516
IF83	ribosomal protein L14	Xenopus laevis	CAA28682	59	2.00E-18	54	79 / 48	2	312
IF84	ubiquitin conjugating enzyme E2L	Homo sapiens	NP03338	268	4.00E-71	82	94 / 140	1	792
IF87	large subunit ribosomal protein rpL44	Aedes triseriatus	AAF87576	172	1.00E-42	74	81 / 104	1	369
IF91	40s ribosomal protein s7	Xenopus laevis	P02362	280	9.00E-75	71	84 / 190	1	631
IF93	ribosomal protein s12	Homo sapiens	XP_004351	202	3.00E-51	78	85 / 120	2	558
IF103	ribosomal protein S28	Cricetulus griseus	AAG49498	95	3.00E-19	86	97 / 51	1	302
IF109	60s ribosomal protein L10	Drosophila melanogaster	O61231	350	7.00E-96	88	95 / 177	1	571
IF112	ribosomal protein L39	Homo sapiens	NP_000991	89	9.00E-18	80	88 / 47	3	246
IF113	translationally controlled tumor protein homolog	Drosophila melanogaster	Q9VGS2	141	3.00E-33	56	73 / 139	1	461
IF114	translationally controlled tumor protein homolog	Drosophila melanogaster	Q9VGS2	148	4.00E-35	56	72 / 139	1	577
IF116	60s ribosomal protein L13	Gallus gallus	P41125	176	1.00E-43	60	72 / 150	3	465
IF117	ribosomal protin eL12	Artemia sp.	CAA26480	100	2.00E-20	77	89 / 77	2	406
IF130	ribosomal protein S12	Homo sapiens	XP_004351	208	5.00E-53	78	85 / 124	2	557
IF137	ribosomal protein L8	Drosophila melanogaster	AAF06828	125	8.00E-29	85	87 / 67	2	239

VIII. *V. harveyi*-infected *P. monodon* ESTs homologues gene expression and protein synthesis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF144	60s ribosomal protein L39	<i>Drosophila melanogaster</i>	O16130	94	3.00E-19	80	87 / 51	1	269
IF148	ribosomal protein S15	<i>Homo sapiens</i>	NP_001009	160	6.00E-39	91	93 / 80	3	316
IF152	ribosomal protein L35A	<i>Homo sapiens</i>	AAH01037	158	2.00E-38	67	79 / 108	2	381
IF154	60s ribosomal protein L5A	<i>Xenopus laevis</i>	P15125	65	2.00E-10	57	74 / 52	1	270
IF156	Elongation factor 1-alpha	<i>Schizophyllum commune</i>	O42820	272	2.00E-72	81	89 / 154	2	589
IF161	ribosomal protein S15	<i>Homo sapiens</i>	NP_001009	160	5.00E-45	91	93 / 80	3	481
IF172	ribosomal protein S11	<i>Homo sapiens</i>	NP_001006	177	5.00E-44	85	91 / 98	-2	311
IF180	40s ribosomal protein S10	Rat	P09900	172	8.00E-50	67	80 / 121	2	540
IF188	Elongation factor 1-alpha F2	<i>Apis mellifera</i>	AAC38958	188	5.00E-83	89	92 / 99	2	573
IF193	transcription co-repressor Sin3	<i>Xenopus laevis</i>	AAD34644	66	6.00E-11	53	81 / 49	2	419
IF195	60s ribosomal protein L37A	<i>Ostertagia ostertagi</i>	O61598	126	1.00E-28	61	71 / 91	1	324
IF200	40s ribosomal protein S11	<i>Xenopus laevis</i>	P41115	238	3.00E-62	77	85 / 153	-1	553
IF202	ribosomal protein S15	<i>Arabidopsis thaliana</i>	CAC05477	123	2.00E-27	69	75 / 91	-2	666
IF205	ribosomal protein L17A	<i>Drosophila melanogaster</i>	JC1253	233	2.00E-68	89	93 / 122	-3	601
IF207	ribosomal protein S16	<i>Homo sapiens</i>	NP_001011	262	4.00E-69	87	96 / 140	-1	620
IF208	60s ribosomal protein L22	<i>Tripneustes gratilla</i>	P13732	101	8.00E-21	49	60 / 105	-3	633
IF210	ribosomal protein S3	<i>Mus musculus</i>	NP_036182	299	2.00E-80	85	89 / 175	1	539
IF213	ribosomal protein L3	<i>Mus musculus</i>	NP_038790	407	E-119	72	86 / 247	2	804
IF215	ribosomal protein S18	<i>Cherax destructor</i>	AAG47944	256	1.00E-67	95	99 / 125	3	504
IF217	60s acidic ribosomal protein P2	Pig	Q29315	58	2.00E-10	65	78 / 43	1	378
IF221	ribosomal protein L24	<i>Homo sapiens</i>	NP_000977	143	1.00E-33	61	70 / 109	2	518
IF232	ribosomal protein L14	<i>Caenorhabditis elegans</i>	T18913	44	1.00E-09	41	63 / 48	2	445
IF234	ribosomal protein S8	<i>Apis mellifera</i>	AAC28863	131	3.00E-30	63	68 / 100	3	351
IF238	ribosomal protein eL12	<i>Artemia sp.</i>	CAA26480	99	3.00E-20	70	86 / 62	2	458

VIII. *V. harveyi*-infected *P. monodon* ESTs homologues gene expression and protein synthesis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF240	ribosomal protein , large P2	Homo sapiens	NP_000995	44	5.00E-06	50	65 / 44	3	403
IF242	40s ribosomal protein S24	Fugu rubripes	O42387	192	2.00E-48	78	87 / 113	2	517
IF243	60s ribosomal protein Po	Drosophila melanogaster	P19889	136	3.00E-33	65	83 / 90	3	441
IF246	40s ribosomal protein S25	Drosophila melanogaster	P48588	128	4.00E-29	75	89 / 78	1	394
IF247	60s ribosomal protein L12	Rattus rattus	P23358	261	7.00E-69	75	89 / 158	2	691
IF270	ribosomal protein S5	Homo sapiens	XP_009371	279	4.00E-74	88	95 / 148	1	601
IF286	RpS9 gene product [alt 2]	Drosophila melanogaster	AAF50249	227	5.00E-77	82	88 / 142	2	613
IF289	ribosomal protein S12	Homo sapiens	XP_004351	199	2.00E-50	78	85 / 124	1	527
IF263	ribosomal protein L18a	Homo sapiens	NP_000971	235	8.00E-64	69	80 / 157	1	569
IF275	60S ribosomal protein L5	styela clava	Q26481	246	2.00E-65	66	79 / 156	1	578
IF281	ribosomal protein S15	Homo sapiens	NP_001009	189	2.00E-47	64	68 / 144	1	470
IF295	ribosomal protein L18a	Homo sapiens	NP_000971	235	8.00E-64	69	80 / 157	1	563
IF298	60S ribosomal protein L8	Aedes albopictus	P41569	346	E-112	81	94 / 182	3	807
IF300	ribosomal protein L24	Homo sapiens	NP_000977	134	7.00E-31	61	68 / 104	2	488
IF315	ribosomal protein L30	Homo sapiens	NP_000980	189	1.00E-47	78	90 / 111	3	411
IF328	60S ribosomal protein L29	Drosophila melanogaster	Q24154	55	3.00E-07	50	66 / 55	2	283
IF332	ribosomal protein L18a	Homo sapiens	NP_000971	226	1.00E-60	69	80 / 157	1	563
IF333	60S ribosomal protein L35a	Xenopus laevis	PO2434	96	8.00E-20	74	78 / 62	2	215
IF338	60s ribosomal protein L37A	Ostertagia ostertagi	O61598	121	3.00E-27	61	71 / 91	2	316
IF362	putative senescence-associated protein	Pisum sativum	BAB33421	130	2.00E-29	82	84 / 74	2	669
IF376	40S ribosomal protein S7	Xenopus laevis	P02362	280	1.00E-74	71	84 / 190	2	685
IF365	QM protein	Bombyx mandarina	AAC98301	399	E-110	81	91 / 179	3	690
IF384	elongation translation factor 1 alpha	Cyanophora paradoxa	AAD03711	301	4.00E-81	69	81 / 210	1	869
IF387	40S ribosomal protein S12	Xenopus laevis	P47840	140	1.00E-32	59	65 / 124	2	857

VIII. *V. harveyi*-infected *P. monodon* ESTs homologues gene expression and protein synthesis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF389	60S ribosomal protein L27a	Tenebrio molitor	Q27021	159	4.00E-38	56	67 / 146	2	866
IF394	40S ribosomal protein S20	Xenopus laevis	P23403	186	2.00E-46	88	95 / 102	3	681
IF432	elongation factor 1 alpha	Salmo salar	AAG38613	243	6.00E-98	82	89 / 140	1	684
IF439	eukaryotic translation initiation factor3	Homo sapiens	XP_010886	57	4.00E-08	60	75 / 45	2	296
IF441	Ef2b gene product	Drosophila melanogaster	AAF57226	232	2.00E-60	83	88 / 134	3	673
IF442	ribosomal protein S17	Homo sapiens	NP_001012	219	9.00E-57	88	94 / 122	3	468
IF450	40S ribosomal protein S20	Xenopus laevis	P23403	186	8.00E-47	88	95 / 102	1	455
IF457	Bmsqd-2	Bombyx mori	BAA07211	131	1.00E-33	78	89 / 76	2	543
IF458	RpL 19 gene product	Drosophila melanogaster	AAF47305	242	1.00E-63	70	79 / 168	3	620
IF466	ribosomal protein S18	Spodoptera frugiperda	AAK92187	246	3.00E-65	88	95 / 134	3	426
IF471	ribosomal protein L29	Spodoptera frugiperda	AAL26577	53	6.00E-12	45	65 / 53	2	330
IF482	ribosomal protein L24	Homo sapiens	NP_000977	132	1.00E-30	61	68 / 105	3	533
IF486	elongation factor-2	Armadillidium vulgare	AAK12341	457	E-133	87	95 / 248	3	820
IF487	ubiquitin and ribosomal protein S27a	Homo sapiens	NP_002945	224	3.00E-58	74	77 / 154	2	792
IF496	60S acidic ribosomal protein P2	Pig	Q29315	79	1.00E-14	61	79 / 65	2	394
IF504	ribosomal protein S5	Mus musculus	NP_033121	257	7.00E-68	71	77 / 192	2	683
IF508	60S ribosomal protein L17	rat	P24049	211	7.00E-57	63	75 / 166	3	588
IF512	eukaryotic translation initiation factor 2 beta subunit	Drosophila melanogaster	P41375	61	5.00E-09	75	77 / 41	1	520
IF514	ribosomal protein L8	Anopheles gambiae	AAD47076	212	6.00E-55	87	91 / 115	3	384
IF521	60S ribosomal protein L5a	Xenopus laevis	P15125	382	E-105	68	79 / 272	3	600

IX. *V. harveyi*-infected *P. monodon* ESTs homologues : Internal / External structure and motility

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF6	INNEXIN INX3	<i>Drosophila melanogaster</i>	Q9VAS7	147	1.00E-34	37	55 / 197	1	
IF30	Myosin regulatory light chain	<i>Drosophila melanogaster</i>	P40423	217	1.00E-69	89	95 / 112	2	536
IF100	Pelota protein	<i>Drosophila melanogaster</i>	AAF52799	212	2.00E-54	56	73 / 172	2	558
IF157	Myosin regulatory light chain	<i>Drosophila melanogaster</i>	P40423	189	3.00E-64	84	92 / 100	1	882
IF196	gamma-actin	<i>Mus musculus</i>	CAA31455	180	1.00E-44	100	100 / 84	1	402
IF274	actin	<i>Tilapia mossambica</i>	CAA77263	160	4.00E-71	100	100 / 76	3	559
IF258	tubulin alpha-1 chain	<i>Homarus americanus</i>	Q25008	422	E-123	88	92 / 219	1	766
IF437	beta - actin	<i>Litopenaeus vannamei</i>	AAG16253	245	E-116	100	100 / 121	1	688
IF460	actin related protein 2/3 complex, subunit3	<i>Homo sapiens</i>	NP_05710	243	1.00E-63	62	80 / 177	3	829
IF505	tubulin alpha-1 chain	<i>Homarus americanus</i>	Q25008	237	E108	97	97 / 116	2	696

X. *V.harveyi*-infected *P. monodon* ESTs homologues : Metabolism

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF1	Guanine nucleotide-binding protein gamma-1	<i>Drosophila melanogaster</i>	P38040	79	3.00E-14	61	77 / 60	1	397
IF2	subunit Vib of cytochrome c oxidase	<i>Saccharomyces cerevisiae</i>	NP_013139	100	1.00E-20	52	70 / 76	2	552
IF4	cytochrome b	<i>Penaeus monodon</i>	NP_038299	363	1.00E-99	85	87 / 213	1	641
IF34	ADP-ribosylation factor 6	<i>Gallus gallus</i>	P26990	355	3.00E-97	95	97 / 170	1	661
IF39	arginine kinase	<i>Callinectes sapidus</i>	AAF43436	159	3.00E-38	92	98 / 76	2	527
IF44	cytochrome b	<i>Penaeus monodon</i>	NP_038299	305	2.00E-82	93	95 / 162	3	508
IF50	unspecific monooxygenase	<i>Nicotiana tabacum</i>	T02995	42	8.00E-07	75	75 / 32	-1	546
IF56	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	NP_038289	442	E-127	92	96 / 234	2	742
IF71	NADH dehydrogenase subunit 1	<i>Penaeus monodon</i>	NP_038300	346	E-104	91	94 / 196	3	676
IF74	PDGF / VEGF - like protein	<i>Drosophila melanogaster</i>	CAC24699	64	2.00E-09	30	52 / 86	2	696
IF85	glutathione s-transferase	<i>Rattus norvegicus</i>	P46418	44	8.00E-04	40	54 / 55	2	370
IF90	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	NP_038289	348	E-100	70	76 / 250	2	813
IF99	glutamyl-tRNA(Glu) amidotransferase	<i>Deinococcus radiodurans</i>	D75346	65	5.00E-10	44	56 / 88	2	557
IF107	unspecific monooxygenase	<i>Nicotiana tabacum</i>	T02995	84	9.00E-19	52	60 / 103	1	689
IF111	ATP synthase oligomycin sensitivity conferral protein precursor	<i>Drosophila melanogaster</i>	Q24439	221	8.00E-57	54	75 / 192	2	758
IF125	ATP synthase FO subunit 6	<i>Penaeus monodon</i>	NP_038292	286	2.00E-76	80	88 / 196	2	747
IF126	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	NP_038289	388	E-107	92	96 / 203	2	612
IF149	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	AAF43374	746	E-110	82	88 / 248	2	746
IF160	Arginase	<i>Xenopus laevis</i>	Q91554	183	2.00E-45	44	65 / 206	3	638
IF171	cytochrome c oxidase subunit II	<i>Penaeus monodon</i>	NP_038290	212	1.00E-55	93	94 / 111	2	407
IF253	ATP synthase oligomycin sensitivity conferral protein precursor	<i>Drosophila melanogaster</i>	Q24439	84	1.00E-15	59	83 / 62	1	471
IF255	Cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	NP_038289	380	E-105	91	94 / 204	2	615

X. *V.harveyi*-infected *P. monodon* ESTs homologues : Metabolism (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF291	amino acid starvation-induced protein	<i>Rattus norvegicus</i>	AAA40765	99	2.00E-33	56	68 / 87	3	534
IF331	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	NP_038289	480	E-135	89	93 / 269	1	809
IF339	ATP synthase FO subunit 6	<i>Penaeus monodon</i>	NP_038292	283	5.00E-78	78	87 / 179	3	802
IF342	SEC61 , gamma subunit	<i>Mus musculus</i>	NP_035473	66	2.00E-10	47	59 / 65	2	353
IF256	cytochrome c oxidase subunit II	<i>Penaeus monodon</i>	NP_038290	209	4.00E-88	73	77 / 144	1	669
IF373	NADH dehydrogenase subunit 4	<i>Penaeus monodon</i>	NP_038296	298	6.00E-80	75	78 / 201	1	611
IF377	ATP synthase oligomycin sensitivity conferral precursor	<i>Drosophila melanogaster</i>	Q24439	158	8.00E-38	54	75 / 133	2	685
IF378	cytochrome c oxidase subunit I	<i>Penaeus monodon</i>	NP_038289	335	1.00E-91	75	82 / 221	3	672
IF418	ATP synthase coupling factor 6, mitochondrial precursor (F6)	<i>Drosophila melanogaster</i>	Q24407	104	1.00E-22	55	73 / 94	3	432
IF454	cytochrome c oxidase subunit 2	<i>Penaeus monodon</i>	NP_038290	356	8.00E-98	82	85 / 223	2	671
IF469	RIKEN	<i>Mus musculus</i>	NP_080731	89	5.00E-18	45	60 / 107	1	432
IF477	2, 4-dienoyl -CoA reductase (NADH)	<i>Rattus norvegicus</i>	S11021	341	7.00E-93	60	73 / 284	2	507
IF501	Na/K-ATPase beta subunit isoform 3	<i>Drosophila melanogaster</i>	AAF17587	110	8.00E-24	38	55 / 157	2	692
IF204	NADH dehydrogenase subunit 6	<i>Penaeus monodon</i>	AAF43384	134	3.00E-32	68	75 / 105	1	385

XI. *V. harveyi*-infected *P. monodon* ESTs homologues : Defense and homeostasis

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF51	Peptidyl-prolyl cis-trans isomerase 5	Caenorhabditis elegans	P52013	206	2.00E-52	68	75 / 153	1	667
IF52	Peptidyl-prolyl cis-trans isomerase 5	Caenorhabditis elegans	P52013	233	1.00E-60	72	78 / 154	2	570
IF62	P lysozyme structural	Mus musculus	NP_038618	101	2.00E-23	51	67 / 98	3	539
IF67	Penaeidin-2 precursor	Penaeus vanamei	NP_81057	66	4.00E-10	49	56 / 67	1	647
IF70	Fc fragment of IgE, low affinity II	Homo sapiens	NP_001993	39	5.00E-07	44	67 / 38	3	533
IF72	11.5kDa antibacterial protein	Carcinus maenas	CAB51030	34	1.00E-04	54	58 / 24	3	435
IF82	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	8.00E-17	37	57 / 95	3	652
IF86	clottable protein	Penaeus monodon	AF089867	472	E-130	97	*/278	-	458
IF98	heat shock - like protein (HSP70)	Ceratitis capitata	AAC23392	75	3.00E-13	83	91 / 37	3	403
IF141	proteinase inhibitor-signal crayfish	Pacifastacus leniusculus	S45677	104	6.00E-22	46	60 / 103	1	503
IF145	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	8.00E-17	37	57 / 95	3	636
IF153	cytosolic manganese superoxide dismutase precursor	Callinectes sapidus	AAF74771	460	E -129	78	86 / 271	3	930
IF165	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	9.00E-17	37	57 / 95	2	672
IF179	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	8.00E-17	37	57 / 95	3	641
IF191	anti-lipopolysaccharide factor	Tachypleus tridentatus	AAK00651	56	2.00E-07	32	48 / 74	1	654
IF219	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	94	2.00E-18	45	64 / 88	1	512
IF220	clottable protein	Penaeus monodon	AF089867	456	E-125	97	*/278	-	450
IF235	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	9.00E-17	37	57 / 95	3	667
IF250	putative VLDL lipoprotein receptor precursor	Mus musculus	NP_062294	50	2.00E-05	30	44 / 85	2	730
IF251	11.5kDa antibacterial protein	Carcinus maenas	CAB51030	64	2.00E-09	40	44 / 81	2	536
IF252	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	8.00E-17	37	57 / 95	1	652
IF261	P lysozyme structural	Mus musculus	NP_038618	120	2.00E-26	52	67 / 113	3	541
IF267	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	1.00E-16	37	57 / 95	1	544
IF272	prophenoloxidase activating factor 3	Bombyx mori	AAL31707	100	1.00E-20	36	53 / 151	1	557

XI. *V. harveyi*-infected *P. monodon* ESTs homologues : Defense and homeostasis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF296	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	88	8.00E-17	37	57 / 95	3	634
IF351	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	83	4.00E-15	43	65 / 81	1	787
IF356	11.5kDa antibacterial protein	Carcinus maenas	CAB51030	34	2.00E-04	54	58 / 24	3	510
IF379	clottable protein	Penaeus monodon	AF089867	480	E-133	98	* / 278	-	452
IF381	Peptidyl-prolyl cis-trans isomerase 5	Caenorhabditis elegans	P52013	210	1.00E-53	68	75 / 151	2	560
IF412	Prophenoloxidase activating factor	Holotrichia diomphalia	CAC12665	91	7.00E-18	60	70 / 64	2	550
IF414	gene MAC25 protein - human	Homo sapiens	I52825	62	9.00E-10	45	53 / 73	2	405
IF419	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	62	3.00E-09	39	45 / 82	2	522
IF433	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	66	2.00E-10	40	45 / 79	1	687
IF438	gene MAC25 protein - human	Homo sapiens	I52825	62	9.00E-10	45	53 / 73	1	389
IF443	11.5 kDa antibacterial protein	Carcinus maenas	CAB51030	62	3.00E-09	39	45 / 82	1	503
IF445	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	60	3.00E-14	44	59 / 52	1	621
IF446	whey acidic protein	Trichosurus vulpecula	AAK69407	52	9.00E-06	34	49/83	2	746
IF451	heat shock cognate 70 kDa protein	Oncorhynchus mykiss	P08108	264	9.00E-95	74	76 / 187	3	772
IF462	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	85	1.00E-16	37	57 / 95	3	428
IF463	anti-lipopolysaccharide factor	Tachypleus tridentatus	AAK00651	63	5.00E-10	33	49 / 78	2	412
IF464	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	85	1.00E-16	37	57 / 95	1	426
IF470	penaeidin-3c precursor	Litopenaeus vannamei	P81060	91	1.00E-18	59	66 / 59	1	412
IF448	serine protease	Pacifastacus leniusculus	CAB63112	361	4.00E-99	70	79 / 242	2	887
IF481	Penaeidin-3c precursor	Litopenaeus vannamei	P81060	79	2.00E-14	56	64 / 73	3	570
IF492	crustin	Litopenaeus vannamei	AAL36898	172	1.00E-42	59	65 / 144	1	515
IF493	Penaeidin-2 precursor	Litopenaeus vannamei	P81057	50	2.00E-05	50	57 / 52	1	651
IF517	Penaeidin-2 precursor	Litopenaeus vannamei	P81057	50	2.00E-05	50	57 / 52	1	639
IF519	anti-lipopolysaccharide factor	Attantic horseshoe crab	A23931	85	4.00E-16	37	57 / 95	2	645

XI. *V. harveyi*-infected *P. monodon* ESTs homologues : Defense and homeostasis (continued)

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF520	penaeidin-3k	Litopenaeus vannamei	AAK83450	67	9.00E-11	46	54 / 73	1	633
IF494	putative antimicrobial peptide (crustin)	Litopenaeus vannamei	AAL36897	71	9.00E-15	73	78/38	2	785

XII. *V. harveyi*-infected *P. monodon* ESTs homologues : Signalling and communication

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
IF444	gamma-aminobutyric acid (GABA-A) receptor subunit epsilon	Rattus norvegicus	NP_075579	57	1.00E-07	30	45 / 159	1	677
IF250	putative VLDL lipoprotein receptor precursor	Mus musculus	NP_062294	50	2.00E-05	30	44/85	2	730

XIII. *V. harveyi*-infected *P. mpondon* ESTs homologues : Cell division / DNA synthesis, repair and replication

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives %	Frame	Sequence length (bp)
IF183	H3 histone	Homosapiens	XP_010903	203	2.00E-51	100	100	3	816
IF233	histone 1	Mytilus edulis	CAA11812	100	3.00E-20	65	75	1	542
IF292	similar to H3 histone, family 3A	Homo sapiens	XP_010903	207	9.00E-63	96	96	1	827
IF420	lated histone H1	Lytechinus pictus	P06144	55	9.00E-07	48	52 / 70	2	820
IF453	histone 1	Mytilus edulis	CAA11812	96	5.00E-19	65	75 / 76	3	871

XIV. *V. harveyi*-infected *P.monodon* ESTs homologues : Non identified function

Clone No.	Genes	Closest species	Accession No.	Score	E value	Identities %	Positives% / length aa	Frame	Sequence length (bp)
IF27	Hypothetical 36.9 kDa protein	Caenorhabditis elegans	P34276	77	1.00E-13	47	63 / 78	2	483
IF29	hypothetical protein	Homo sapiens	CAB66819	45	4.00E-04	50	57 / 52	3	463
IF37	KIAA 1594 protein	Homo sapiens	BAB13420	93	1.00E-18	34	60 / 127	2	372
IF218	hypothetical protein FJ12878	Homo sapiens	NP_071349	226	2.00E-58	45	58 / 280	1	845
IF123	unnamed protein product	Mus musculus	BAC30584	47	2.00E-04	32	51/70	3	616
IF131	ebiP7015	Anopheles gambiae	EAA1227	166	3.00E-40	67	84/113	1	805
IF360	CG14206 gene product	Drosophila melanogaster	AAF48978	165	4.00E-48	71	79/112	3	543
IF272	BcDNA GH02921	Drosophila melanogaster	AAD38586	104	2.00E-21	36	52/149	1	557
IF364	CG6001 gene product	Drosophila melanogaster	AAF56679	382	E-105	72	85/257	2	922
IF320	CG9354 gene product	Drosophila melanogaster	AAF54369	127	5.00E-29	68	73/87	2	386
IF187	CG8444 gene product	Drosophila melanogaster	AAF54350	70	2.00E-11	54	59/59	3	636
IF201	PTD007 protein	Homo sapiens	NP_054769	71	2.00E-11	36	51/97	1	568
IF236	p105 coactivator	Rattus norvegicus	NP_073185	79	5.00E-14	49	72/69	2	764
IF336	AK012526 putative	Mus musculus	BAB28297	45	4.00E-06	52	65/44	1	362
IF350	AK002940 putative	Mus musculus	BAB22470	167	6.00E-41	62	78/129	3	423
IF244	hypothetical protein F26E4.9	Caenorhabditis elegans	T21416	100	1.00E-20	47	70/90	1	132
IF306	hypothetical protein; CGI-117 protein	Homo sapiens	NP_057475	61	1.00E-08	60	81/38	3	519
IF502	hypothetical protein 51.8 kDa protein	Leishmania major	CAC59772	47	1.00E-04	39	62/43	3	586
IF307	RE63456p	Drosophila melanogaster	AAL49199	129	7.00E-30	70	75/91	3	367

Appendix B

I. ANOVA test of ALF expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3454.944	5	690.989	5.114	.010
With Groups	1621.333	12	135.111		
Total	5076.278	17			

II. Dancan test of ALF expression.

Dancan^a

Hours	N	Subset for alpha =.05		
		1	2	3
0	3	72.67		
48	3	90.67	90.67	
24	3		101.33	101.33
3	3		104.00	104.00
12	3		106.67	106.67
6	3			116.33
Sig.		.082	.144	.168

Means for groups in homogeneous subsets are displayed.

^a Uses Harmonic Mean Sample Size = 3.000.

III. ANOVA test of HSP 90 expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	447.111	5	89.422	3.787	.027
With Groups	283.33	12	23.611		
Total	730.44	17			

IV. Dancan test of HSP 90 expression.

Dancan^a

Hours	N	Subset for alpha =.05	
		1	2
0	3	28.33	
48	3	30.00	
3	3	36.00	36.00
24	3	36.00	36.00
6	3		40.00
12	3		42.33
Sig.		.098	.184

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

V. ANOVA test of lysozyme expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2678.44	5	535.689	4.407	.016
With Groups	1458.67	12	121.556	.	
Total	4137.11	17	.		

VI. Dancan test of lysozyme expression.

Dancan^a

Hours	N	Subset for alpha =.05		
		1	2	3
0	3	38.33		
3	3	46.00	46.00	
6	3		60.00	60.00
12	3		65.33	65.33
24	3		66.33	66.33
48	3			73.33
Sig.		.411	.058	.195

Means for groups in homogeneous subsets are displayed.

^a Uses Harmonic Mean Sample Size = 3.000.

VII. ANOVA test of crustin expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10578.27	5	2115.656	19.938	.000
With Groups	1273.33	12	106.111		
Total	11851.61	17			

VIII. Dancan test of crustin expression.

Dancan^a

Hours	N	Subset for alpha =.05		
		1	2	3
3	3	41.00		
6	3	43.67		
12	3	51.33		
24	3		72.67	
48	3		88.33	
0	3			106.67
Sig.		.265	.087	1.000

Means for groups in homogeneous subsets are displayed.

^a Uses Harmonic Mean Sample Size = 3.000.

IX. ANOVA test of penaeidin expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16472.94	5	3294.589	15.464	.000
With Groups	2556.67	12	213.056		
Total	19029.61	17			

X. Dancan test of crustin expression.

Dancan^a

Hours	N	Subset for alpha =.05			
		1	2	3	4
12	3	48.33			
6	3	57.00	57.00		
3	3		76.00		
24	3		79.33		
48	3			107.67	
0	3				137.33
Sig.		.481	.099	1.000	1.000

Means for groups in homogeneous subsets are displayed.

^a Uses Harmonic Mean Sample Size = 3.000.

XI. ANOVA test of SPI expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	451.833	5	90.367	1.315	.321
With Groups	824.667	12	68.722		
Total	1276.50	17			

XII. Dancan test of SPI expression.

Dancan^a

Hours	N	Subset for alpha = 0.5
6	3	69.33
3	3	76.33
48	3	79.00
12	3	81.67
24	3	81.67
0	3	85.00
Sig.		.058

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

XIII. ANOVA test of ProPO expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	331.833	5	66.367	.459	.800
With Groups	1736.667	12	144.722	.	
Total	2068.500	17	.		

XIV. Dancan test of ProPO expression.

Dancan^a

Hours	N	Subset for alpha = 0.5
3	3	43.00
6	3	46.33
12	3	49.00
48	3	51.33
0	3	53.67
24	3	55.67
Sig.		.265

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

XV. ANOVA test of HSP 70 expression.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	93.111	5	18.622	.189	.961
With Groups	1183.333	12	98.611		
Total	1276.444	17			

XVI. Dancan test of HSP 70 expression.

Dancan^a

Hours	N	Subset for alpha = 0.5
6	3	87.33
48	3	88.33
24	3	89.67
12	3	92.00
3	3	92.33
0	3	93.67
Sig.		.490

Means for groups in homogeneous subsets are displayed.

^a Uses Harmonic Mean Sample Size = 3.000.

Appendix C

Identification of Immune-Related Genes in Hemocytes of Black Tiger Shrimp (*Penaeus monodon*)

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Abstract: An expressed sequence tag (EST) library was constructed from hemocytes of the black tiger shrimp (*Penaeus monodon*) to identify genes associated with immunity in this economically important species. The number of complementary DNA clones in the constructed library was approximately 4×10^5 . Of these, 615 clones having inserts larger than 500 bp were unidirectionally sequenced and analyzed by homology searches against data in GenBank. Significant homology to known genes was found in 314 (51%) of the 615 clones, but the remaining 301 sequences (49%) did not match any sequence in GenBank. Approximately 35% of the matched ESTs were significantly identified by the BLASTN and BLASTX programs, while 65% were recognized only by the BLASTX program. Of the 615 clones, 55 (8.9%) were identified as putative immune-related genes. The isolated genes were composed of those coding for enzymes and proteins in the clotting system and the prophenoloxidase-activating system, antioxidative enzymes, antimicrobial peptides, and serine proteinase inhibitors. Three full-length ESTs encoding antimicrobial peptides (antilipopolysaccharide and penaeidin homologues) and a heat shock protein (cpn10 homologue) are reported.

Key words: shrimp immunity, *Penaeus monodon*, EST, hemocytes.

INTRODUCTION

Among cultured marine shrimps the most economically important species is the black tiger prawn *Penaeus monodon*. Farmed *P. monodon* contributes over 50% of the total world shrimp production annually (Rosenberry, 2000). Shrimp

farming has attained great economic and social importance in Thailand as a source of income and employment. However, this industry has consistently suffered great production losses from the outbreak of infectious diseases, particularly from viruses and bacteria. Sustainability of the shrimp industry depends largely on efficient disease control and on shrimp health. Therefore, disease prevention has been considered a priority for this industry (Roch, 1999).

Genetic selection and management of broodstock and seed production are important areas of research to increase

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efficiency in the culture and management of *P. monodon*. Accordingly, genetic diversity and effects of hatchery-produced stock on wild populations of *P. monodon* in Thailand have been extensively investigated (Tassanakajon et al., 1997, 1998; Supungul et al., 2000; Klinbunga et al., 2001). Our findings on patterns of genetic differentiation of *P. monodon* in Thailand have enabled us to domesticate this economically important species, and two separated full-sib groups of *P. monodon* from Trat and Satun are currently being cultured in our laboratory (Jarayabhand et al., 1998).

At present several serious diseases found in commercially cultured *P. monodon* are still not well understood or controllable. Development of *P. monodon* stocks that are resistant to either disease (e.g., have specific pathogen resistance [SPR]) or environmental stress, or both is crucial for this industry and could serve as an alternative source of broodstock for commercial purposes. Knowledge of shrimp immunity is necessary to provide the means to control and minimize the loss of production due to infectious diseases. Moreover, identification and characterization of immune-related genes and proteins and their responses to infections can be applied to the selection of *P. monodon* that are resistant to disease and environmental stress.

Although arthropods have not acquired adaptive immune systems, an efficient innate immunity against microbial infection has been reported (Lemaitre et al., 1997). Cell defense mechanisms in invertebrates are closely associated with humoral reactions to eliminate invading microorganisms (Söderhäll et al., 1996). In crustaceans the major immune response is carried out by hemolymph, which contains different types of hemocytes (Iwanaga et al., 1998; Johansson et al., 2000). These hemocytes are involved in several defense reactions including recognition, phagocytosis, encapsulation, cytotoxicity, and melanization (Söderhäll and Cerenius, 1992; Johansson et al., 2000). Several proteins and peptides are synthesized and stored in hemocytes and released into the hemolymph upon infection. In the horseshoe crab *Limulus polyphemus*, storage peptides (e.g., antilipopolysaccharide [anti-LPS], clotting factors, and antimicrobial substances) are released into plasma upon stimulation by microbial substances such as lipopolysaccharides and β -glucans (Iwanaga et al., 1998).

Tissue-specific expression based on expressed sequence tags (ESTs) of cephalothorax, eyestalk, and pleopods of *P. monodon* has been reported (Lehnert et al., 1999). Forty-nine newly isolated genes that had not been identified in crustaceans were reported. Recently, several immune genes have been discovered in other *Penaeid* shrimps, *Litopenaeus*

vannamei, *L. setiferus* (Gross et al., 2001), and *P. japonicus* (Rojtinakorn et al., in press). Likewise, to identify genes associated with immunity in normal hemocytes of the black tiger shrimp *P. monodon*, we generated ESTs from the hemocyte transcripts of *P. monodon*. Several putative immune-related genes were isolated and partially characterized. The sequences of 3 full-length ESTs coding for antimicrobial peptides (anti-LPS and penaeidin homologues) and a small heat-shock protein (cpn10 homologue) are reported.

MATERIALS AND METHODS

Hemocytes and Total RNA Preparation

Hemolymph was collected from broodstock-sized *P. monodon* using 10% sodium citrate as an anticoagulant. Hemocytes were separated from plasma by centrifugation at 800 g for 10 minutes at room temperature. Total RNA was immediately prepared using TRIzol reagent (Gibco BRL, Rockville, Md.) and kept in 70% ethanol at -80°C until needed.

Preparation of mRNA and Synthesis of cDNA

The messenger RNA of *P. monodon* hemocytes was prepared using a QuickPrep mRNA Purification Kit (Amersham Pharmacia Biotech, Piscataway, N.J.). Five micrograms of the poly(A)⁺ RNA was subjected to complementary DNA synthesis using a Time Saver cDNA Synthesis Kit (Amersham Pharmacia Biotech). An *EcoRI*/*NotI* adapter was ligated to each end of the cDNA. This adapter is composed of 2 oligomers forming a duplex with a blunt end and an *EcoRI* overhang for insertion of cDNA into the *EcoRI* site of λ -ZAPII vector.

Construction of Hemocyte cDNA Library

A hemocyte cDNA library was constructed using λ -ZAPII vector (Stratagene, La Jolla, Calif.). *In vivo* excision to convert λ clones into pBluescript clones was carried out. The plasmid was then transformed into *Escherichia coli* SOLR following a protocol recommended by the manufacturer (Stratagene). The transformants were selected on 2xYT plates. Plasmid DNA was prepared using a GFX micro Plasmid Prep Kit (Amersham Pharmacia Biotech). The insert size of each clone was electrophoretically

determined after digestion with *EcoRI* following standard protocols (Sambrook et al., 1989). Only clones containing inserts larger than 500 bp were subjected to further analysis.

DNA Sequencing and Data Analysis

Sequencing based on dideoxy chain termination was carried out using a ThermoSequenase Fluorescent Labelled Primer Sequencing Kit (Amersham Pharmacia Biotech) with M13 forward and reverse primers on an automated DNA sequencer LC4000 (LI-COR, Lincoln, Neb). Data were compared with those deposited in GenBank using the BLASTN and BLASTX programs (NCBI Advanced Blast Search; available at <http://www.ncbi.nlm.nih.gov>). Significant probabilities and numbers of matched nucleotides and proteins were considered when *E* values were less than 10^{-4} and a match of more than 100 nucleotides for BLASTN and a match of more than 10 amino acid residues for BLASTX were present.

RESULTS AND DISCUSSION

The EST library was successfully constructed to survey immune-related genes expressed in hemocytes of *P. monodon*. The number of EST clones in the library was approximately 4×10^5 . This number was sufficient to cover the predominantly expressed mRNA in hemocytes (Adams et al., 1991). Randomly selected clones were examined for their insert length to avoid sequencing small inserts or empty clones. Only clones containing relatively large inserts (≥ 500 bp) were sequenced.

We sequenced 615 ESTs. Partial nucleotide sequences were analyzed by homology searches against data in GenBank for nucleotide similarity (BLASTN) and similarity of translated protein sequences (BLASTX) (Altschul et al., 1990). A putative function of each EST was assigned according to the most significant similarity of a particular EST with that of the matched gene in GenBank.

In total 314 (51%) of the clones represented gene homologues, whereas the remaining clones (49%) did not match any sequence in Genbank. Of the matched EST 109 (35%) were identified as known genes by the BLASTN and BLASTX programs, while the remaining 205 (65%) of the clones were recognized only by the BLASTX program. This was not unexpected because of the limited number of nucleotides and proteins of *Penaeid* shrimps and their closely related species deposited in GenBank. Multiple examples of

Table 1. Number and Percentage of Identified *Penaeus monodon* ESTs by Functional Category

Functional category	Number in category	%EST
Metabolism	71	11.5
Gene expression and protein synthesis	109	17.7
Cell division/DNA replication and repair	6	1.0
Internal/external structure and motility	46	7.5
Signaling and communication	9	1.5
Defense and homeostasis	55	8.9
Unidentified function	18	2.9
Unknown	301	49.0
Total	615	100

mitochondrial 16S ribosomal RNA genes and cytochrome oxidase were found. The presence of mitochondrial sequences in our EST library indicated contamination of hemocytes with mitochondrial transcript, which probably resulted from the high A + T content of the *P. monodon* mitochondrial genome. Contamination of mitochondrial sequences was previously found in *P. monodon* EST libraries established from pleopods and cephalothorax (Lehnert et al., 1999).

The EST homologues were classified into broad functional categories based on significant sequence homology according to Adams et al. (1991). The number of ESTs in each of the functional categories was summarized (Table 1). The ESTs encoded homologues of proteins whose genes have been described in different organisms including *Penaeid* shrimps, other crustaceans, *Drosophila*, human, mouse, and chicken. A number of the ESTs had significant homology with several proteins described in *Drosophila* (*E* values = 10^{-9} to 10^{-141}), although many did not show the highest homology with the fly proteins. Fifty-five (8.9%) of the clones were gene homologues involved in defense mechanisms (Table 2, accession number BIO18071–18100). These ESTs encode putative components of the clotting system (glutamine γ -glutamyl transferase, hemocyte protease), the prophenoloxidase system (proPO and its activating enzymes), antioxidative enzymes (peroxidase and catalase), antibacterial peptides (anti-LPS factor, penaeidins, 11.5-kDa antibacterial peptide, and lysozyme), and serine proteinase inhibitors. ESTs encoding 3 heat shock protein homologues, hsp10 (also called cpn10), hsp70, and hsp 90, were also identified.

Table 2. *Penaeus monodon* EST Homologues Classified into Defense and Homeostasis Groups

Accession no.	Genes	Closest species	Accession no. of closest species	E value	Matched (%)	Length (bp)	Frequency
BIO18071	Antilipoplysaccharide factor	Atlantic horseshoe crab	A23931	9.00E-16	65	674	5
BIO18072, BIO18073, BIO18074	11.5-kDa Antibacterial protein	<i>Carcinus maenas</i>	CABS51030	2.00E-10	65	683	5
BIO18075, BIO18076, BIO18077, BIO18078	Proteinase inhibitor-signal crayfish	<i>Pacifastacus leniusculus</i>	S45677	5.00E-31	66	653	7
BIO18079, BIO18080	Penaedine-3c precursor	<i>Penaeus vannamei</i>	P81060	2.00E-15	90	594	6
BIO18081	P lysozyme structural	Mouse	NPO386i8	1.00E-21	66	684	2
BIO18082, BIO18083, BIO18084	Hemocyte protein-glutamine γ -glutamyltransferase	<i>Tachypleus tridentatus</i>	Q05187	2.00E-48	76	746	3
BIO18085	Thymosin β -11	<i>Oncorhynchus mykiss</i>	CAA21832	4.00E-27	64	872	3
BIO18086	Thymosin β -9 and β -8	Bovine	P21752	2.00E-04	77	706	2
BIO18087	Pro-phenoloxidase activating enzyme-I precursor	<i>Holotrichia diomphalia</i>	BAA34642	3.00E-06	64	340	1
BIO18088	Protein-kinase c inhibitor	Bovine	A35350	2.00E-38	78	756	1
BIO18089	Penaedin-2 precursor	<i>Penaeus vannamei</i>	P81057	1.00E-51	57	465	1
BIO18090	Prophenoloxidase	<i>Penaeus monodon</i>	AAD45201	1.00E-51	80	465	1
BIO18091	Glutathione peroxidase	Human	A45207	3.00E-18	76	331	1
BIO18092	Peroxidase	<i>Aedes aegypti</i>	AAC97504	1.00E-21	52	747	1
BIO18093	Protein-glutamine γ -glutamyltransferase	<i>Tachypleus tridentatus</i>	A45321	2.00E-35	76	357	1
BIO18094	Heat shock cognate 70 kDa	<i>Trichoplusia ni</i>	1495233	3.00E-36	95	298	1
BIO18095, BIO18096	Heat shock protein 90	Chicken	HHCH90	9.00E-27	70	618	2
BIO18097	Heat shock protein 70	<i>Hydra magripapillata</i>	Q05944	2.00E-56	90	806	1
BIO18098	Hemocyte protease-1	<i>Manduca sexta</i>	AAB94557	1.00E-12	57	700	1
BIO18099	Protein c	Mouse	NP032960	3.00E-10	63	708	1
BIO18100	Heat shock protein 10	<i>Gallus gallus</i>	AAB86581	2.00E-25	73	665	1

The open reading frame (ORF) of each clone was identified using the Genetyx program (ABI). Three full-length ESTs were found, 2 of which were homologues of antimicrobial effectors (anti-LPS factor and penaedin), while the 3rd-clone was an hsp 10 (cpn10) homologue.

The clone (accession number BIO18071) that encoded a homologue of anti-LPS factor contained an ORF of 372 bp encoding a 123 amino acid protein (Figure 1). An anti-LPS factor was previously reported in the Atlantic horseshoe crab (*Limulus polyphemus*) and the Japanese horseshoe

crab (*Tachypleus tridentatus*). The existence of anti-LPS has not been reported in any *Penaeus* species and thus is first reported in *P. monodon*. The deduced amino acids showed 65% homology with those of *L. polyphemus*. It is a cationic protein naturally found in hemolymph of horseshoe crabs that binds and neutralizes bacterial endotoxin (LPS) and has a strong antibacterial effect, especially on the growth of gram-negative R-type bacteria (Morita et al., 1985; Wainwright et al., 1990). The anti-LPS factors from *L. polyphemus* and *T. tridentatus* and their homologue from

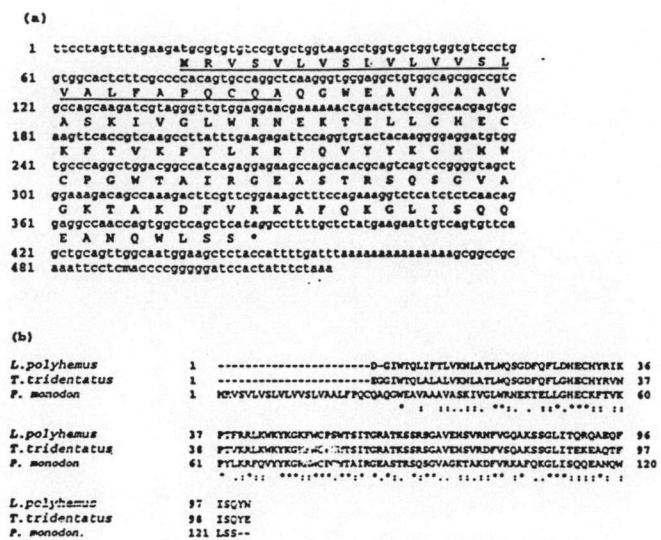


Fig. 1. a: Nucleotide and deduced amino acid sequences of the putative anti-LPS factor in *P. monodon*. An asterisk indicates the stop codon. The underlined sequence corresponds to the putative signal peptide predicted by SignalP version V1.1 software. b: Sequence alignment of deduced amino acids of a newly isolated anti-LPS homologue in *P. monodon* with anti-LPS factors from horseshoe crabs *L. polyphemus* and *T. tridentatus* (Muta et al., 1987; Aketagawa et al., 1986).

P. monodon showed 2 conserved cysteine residues and a highly conserved sequence of a cluster of positively charged residues within the disulfide loop (Figure 1, b), which are necessary for LPS binding to the lipid A moiety (Aketagawa, et al., 1986, Hoess et al., 1993). The amino-terminal domain of the putative anti-LPS factor in *P. monodon* contained an extra 23 amino acid residues of a signal peptide necessary for transportation of this protein. Regardless of the signal peptide, the amino terminus of the putative *P. monodon* anti-LPS was also hydrophobic, similar to those of *L. polyphemus* and *T. tridentatus*.

The other full-length EST (accession number BIO18089) contained an ORF of 225 bp encoding 74 amino acid residues (Figure 2). The deduced amino acid sequence of this EST revealed highly significant homology with antimicrobial peptides (penaeidins 1, 2, and 3a) found in *P. vannamei* (Figure 2, b). These peptides showed a conserved NH₂-terminal proline-rich domain and a COOH-terminal domain containing 6 cysteine residues that had been reported earlier to be engaged in 3 disulfide bridges (Bachère et al., 2000). However, this EST did not contain the medial sequence of 6 amino acids (Gly-Pro-Ile-Gly-Pro-Tyr) that links 2 conserved domains found in penaeidin 3a (amino acid residues 43–48) but not in penaeidins 1 and 2. As in previously reported penaeidins, the 2 organized doublets of

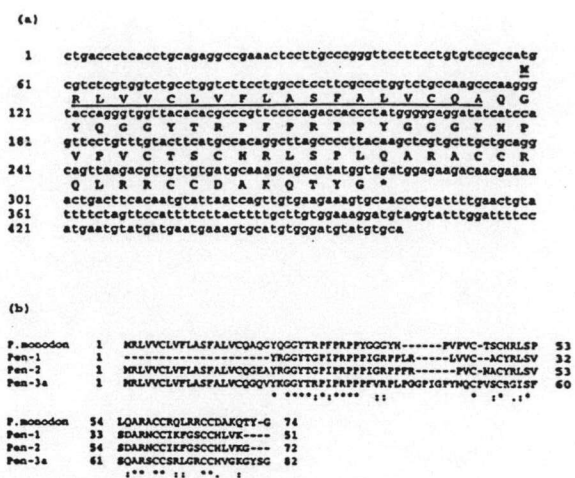


Fig. 2. a: Nucleotide and deduced amino acid sequences of the penaeidin homologue in *P. monodon*. An asterisk indicates the stop codon. The underlined sequence corresponds to the putative signal peptide predicted according to the sequence similarity to *P. vannamei* penaeidins. b: Sequence alignment of deduced amino acids of *P. monodon* penaeidin homologue with penaeidins 1, 2, and 3c from the white shrimp *P. vannamei* (Destoumieux et al., 1997; Bachère et al., 2000).

cysteine residues were separated by 5 amino acid residues in *P. monodon* (Arg-Gln-Leu-Arg-Arg, amino acid residues 61–65). The centralmost cysteine residues were separated by 2 amino acid residues as in penaeidin 2, rather than 1 or 3 amino acid residues as found in penaeidins 1 and 3a, respectively (Destoumieux et al., 1997).

The putative *P. monodon* penaeidin contained a signal peptide of 19 residues almost identical to those of *P. vannamei*. Destoumieux et al. (1997) have proposed that a cleavage site for signal peptidase in the deduced amino acid sequence of penaeidin 3 is located after glycine preceding glutamine at position 1, but we found alanine followed by glutamine at identical positions. This suggested the occurrence of a single mutation from G (*P. vannamei*) to C (*P. monodon*) at the 2nd position of the codon. The final glycine codons found in penaeidin 2 (amino acid residue 72) and penaeidin 3a (amino acid residue 82) of *P. vannamei* were also observed in the newly isolated penaeidin homologue in *P. monodon*. The conserved Pro-Arg-Pro motif (amino acid residues 31–33), which is present in the proline-rich antimicrobial peptide family from insects (Hetru et al., 1998) and penaeidins in *P. vannamei* (Destoumieux et al., 1997), was also found.

Penaeidins have a broad spectrum of antimicrobial properties including fungicidal activity and strong antibacterial activity against gram-positive bacteria (Desto-

umieux et al., 1999). The high homology between the putative *P. monodon* penaeidin and well-characterized penaeidins of *P. vannamei* suggests they may have similar antimicrobial activities. Two posttranslational modifications have been found in penaeidin 3 of *P. vannamei* including blockage of the NH₂ terminus by a pyroglutamic acid residue and amidation of the COOH terminus (Destoumieux et al., 1997). Whether similar posttranslational modifications also occurred in the penaeidin homologue of *P. monodon* cannot be concluded because this antibacterial peptide has not yet been characterized in this species.

The complete ORF (accession number BIO18100) coding for a homologue of a small heat shock protein (cpn10) contained nucleotide sequences of 309 bp, encoding a 102 amino acid protein (Figure 3). Heat shock proteins play an important role during stress (depressed ATP levels, oxygen depletion, decreased intracellular glucose levels). Denaturation of proteins in the cell triggers the synthesis of heat shock proteins intracellularly (Lau et al., 1997; Tom et al., 1999). Under stress conditions they were found to prevent stress-induced protein aggregation and to improve the regeneration of proteins denatured by thermal stress. Comparing the deduced amino acids of this 10-kDa heat shock protein with those of other vertebrates revealed high homology of the entire cpn10 sequences (73% to 75%) across distantly related organisms. The putative cpn10 of *P. monodon* has not been isolated in invertebrates at either the DNA or the protein level and thus is first reported in *P. monodon*.

Previous studies indicated that the mammalian cpn10, present in the mitochondria, is nuclearly coded. For full chaperonin function, cpn10 forms a complex with another distinct family member, cpn60 (Fayet et al., 1989; Dickson et al., 1994). This chaperonin complex is the primary site for protein folding of multimeric enzyme complexes and is necessary for folding of newly synthesized or translocated proteins.

The cpn10 homologue contained a positive net charge of one (15 cationic residues and 14 anionic residues). Hartman et al. (1992) compared partial Hsp10 sequence of *Rattus norvegicus* with Cpn10 from 7 bacterial species and indicated identical residues and conservative replacement defined as K/R, T/S, D/E, Q/E, D/N, and I/L. All except the T/S and D/N replacements were observed when the putative cpn10 of *P. monodon* was compared with that of *Homo sapiens*, *Mus musculus*, and *Gallus gallus*.

Results from EST analysis indicate the potential of this molecular approach for isolation of new uncharacterized

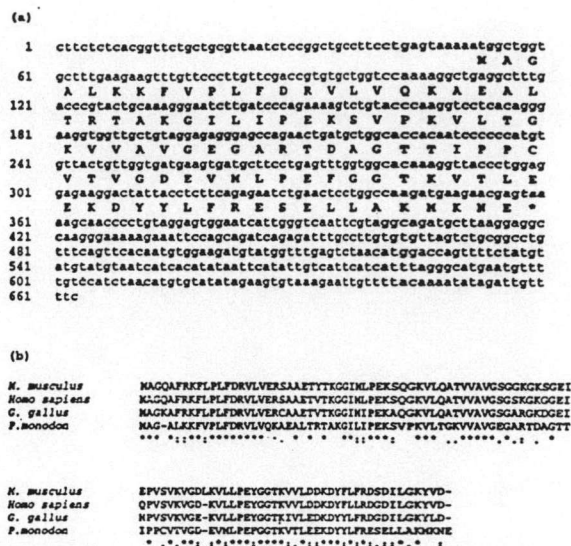


Fig. 3. a: Nucleotide and deduced amino acid sequences of the cpn10 homologue in *P. monodon*. An asterisk indicates the stop codon. b: Sequence alignment of deduced amino acids of *P. monodon* cpn10 homologue with other mammalian cpn10s (Dickson et al., 1994).

gene homologues in *P. monodon*, for which the number of well-characterized genes is limited. Three full-length ESTs found in the present study can be expressed and further characterized to confirm their functional activities at the protein level.

ACKNOWLEDGEMENTS

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Immune-related genes in haemocytes of the black tiger shrimp, *Penaeus monodon*, infected with *Vibrio harveyi*

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SUMMARY: A cDNA library was constructed from haemocytes of the black tiger shrimp *Penaeus monodon* that were infected with the luminous bacteria, *Vibrio harveyi*, to survey the immune-related genes which are induced and expressed in haemocytes. Four hundred and nine cDNA clones were sequenced and subjected to homology searches in the DNA databases. One hundred and eighty one clones (44.3%) showed significant homology to known genes but the remaining sequence (55.7%) did not match to any sequence in the GenBank. Forty one clones (10%) were identified as putative immune-related genes and represented 14 different immune genes. Antimicrobial peptides were the most common group of immune-related ESTs found in infected haemocytes. The ESTs coding for putative anti-lipopolysaccharide factor (16 clones) and 11.5 kDa antibacterial protein (8 clones) were predominate among the immune genes indicating the high abundance of these transcripts in infected hemocytes. High expression of these antimicrobial proteins suggests that they play a major role in response to *V. harveyi* infection.

KEY WORDS: *Penaeus monodon*, immune genes, haemocytes, *Vibrio harveyi*, EST

INTRODUCTION

The black tiger shrimp, *Penaeus monodon*, is the most important *Penaeid* species that is cultured throughout the Southern Indo Pacific region. Farming of *P. monodon* has accomplished a great economic and social importance in the region constituting a significant source of income and employment. Despite the success of shrimp farming industry, the problem due to outbreaks of infectious diseases has become more serious causing a great loss to the industry as high as hundreds of million dollars per year. Infectious diseases in *P. monodon* are caused mainly by white-spot syndrome virus (WSSV), yellow-head virus (YHV) and luminescent bacteria, *Vibrio* species. *V. harveyi* has been described in outbreaks of vibriosis in the black tiger shrimp culture in Taiwan, the Philippines and Thailand.^{1,2,3,4} The previous reports have shown the efficacy of vaccination against vibriosis^{5,6} and the survival of larvae by addition of killed *Vibrio* cells in micro-encapsulated diet⁷. However, effective prevention and control of infectious diseases requires knowledge of shrimp immunity for a better understanding of immune reactions against invading pathogens.

Immune reactions in crustaceans occurred mainly in haemolymph. Several proteins and peptides are

synthesized and stored in haemocytes and haemolymph plasma.^{8,9} Defense mechanisms in crustacean haemocytes include blood coagulation, melanization, agglutination, antimicrobial activity, encapsulation and phagocytosis.^{10,11}

Prophenoloxidase (proPO) cascade is the most understood immune mechanism. The components of microbial cell surfaces such as β -glucan, lipopolysaccharide and peptidoglycan, mediate the recognition molecules leading to the activation of proPO cascade.^{12,13,14} Upon microbial infection, degranulation of haemocytes is also activated leading to the release of specific proteins including several clotting factors essential for haemolymph coagulation and a large number of antimicrobial substances.^{15,16}

Expressed Sequence Tag (EST) approaches have been successfully used for the discovery of immune genes in penaeid shrimps including *Litopenaeus vannamei*, *L. setiferus*¹⁷ and *P. japonicus*.¹⁸ In this study, we used the EST analysis to identify immune genes expressed in haemocytes of *V. harveyi* infected *P. monodon* that were significantly induced and expressed in response to bacterial infection.

MATERIALS AND METHODS

Haemocyte and total RNA preparation

Sub-adult shrimp, *P. monodon* was infected with 10^7 CFU/ml *Vibrio harveyi* 1526 (kindly provided by Shrimp Culture Research Center, Charoenpokaphand Group of Companies). After 48 hours of post-infection, haemolymph was collected by using 10% sodium citrate as an anti-coagulant. Haemocytes from 5 individual shrimps were pooled after the isolation by centrifugation at 800 g for 10 minutes. Total RNA was prepared using TRIzol reagent (Gibco BRL) and kept in 70 % ethanol at -80 °C until needed.

Preparation of mRNA and construction of cDNA library

The mRNA of *V. harveyi* infected shrimp haemocytes was prepared from total RNA using a Quick Prep mRNA Purification kit (Amersham Pharmacia Biotech) and 5 micrograms of that were used for synthesis of cDNA and construction a cDNA library by SuperScript™ Lambda System kit (Gibco BRL) with a *NotI* oligo dT primer. A *Sall* adapter was ligated to both termini of blunt-end cDNA fragments. After *NotI* digestion, the cDNAs had *NotI* termini at one end and *Sall* termini at the other. The cDNAs were size-fractionated by the chromatography column to obtain large cDNAs (>500 bp) and these cDNAs were directly inserted to the *NotI* and *Sall* sites of lambda Zip Lox vector (Stratagene). Conversion of the recombinant lambda vector into the pBluescript plasmid was carried out by *in vivo* excision in DH10B cells. The transformants were selected on LB plates containing ampicillin at a concentration of 100 mg/ml. Recombinant clones were randomly picked and the plasmids were isolated using a GFX™ micro Plasmid Prep kit (Amersham Pharmacia Biotech).

DNA sequencing and data analysis

The dideoxy chain termination-based sequencing was carried out using a ThermoSequenase Fluorescent Labelled Primer Sequencing kit (Amersham Pharmacia Biotech) with M13 primers on automated DNA sequencer LC4000 (LICOR). The sequence of each cDNA was compared with sequence in the nucleotide sequence database at the National Center for Biotechnology Information (NCBI) by the BLASTN and BLASTX programs (<http://www.ncbi.nlm.nih.gov>). Significant probabilities and numbers of matched nucleotides/proteins were considered when E-values < the 10^{-4} and a match > 100 nucleotides for the BLASTN and a match > 10 amino acid residues for the BLASTX, respectively.

RESULTS AND DISCUSSION

A cDNA library of 2.5×10^5 clones was constructed from the mRNA of the black tiger shrimp haemocytes infected with *Vibrio harveyi*. Four hundred and nine clones were randomly picked and partially sequenced. The nucleotide sequences were analyzed by homology search against data in the GenBank based on nucleotide similarity (BLASTN) and similarity of translated protein sequences (BLASTX).¹⁹⁾ A putative function of each EST was assigned according to a similarity greater than 45% based on at least 100 bp and at least 10 amino acid residues by the BLASTN and BLASTX, respectively.²⁰⁾ A total of 181 clones (44%) showed homology to known genes whereas 228 clones (56%) showed no significant homology with any gene in GenBank and were considered to be novel genes or functional unidentified genes. Sixty seven clones (37%) of the matched ESTs were identified as known genes by the BLASTX and BLASTN programs while 114 clones (63%) were only recognized by the BLASTX program.

The significant match may detect a homologue of a gene whose function is well defined. Therefore, we may predict the biological and physiological role for a newly isolated gene. The matched ESTs were classified into broad functional categories based on protein homology, according to the criteria proposed by Adams et al.²¹⁾ The number of ESTs in each of the functional category were shown in Table 1.

Table 1 The number of *P. monodon* ESTs in each of the functional category.

	No. in category	% of EST analysed
Gene expression, regulation, protein synthesis	91	22
Internal/external structure and motility	10	2
Metabolism	31	8
Defense and homeostasis	41	10
Signaling and communication	3	1
Cell division/DNA synthesis, repair, replication	5	1
Total matched ESTs	181	44
Unmatched ESTs	228	56
cDNAs sequenced	409	100

Forty-one clones, representing 10% of the total clones sequenced, are putative immune genes. These clones represent 14 different immune genes (Table 2, accession no. BI784440-BI784460). Among these putative immune genes, the majority are antimicrobial peptides/proteins including anti-lipoplysaccharide factor, 11.5 kDa antibacterial protein, penaeidin and lysozyme. Other immune-related genes are prophenoloxidase activating factor, protease inhibitors (proteinase inhibitor, gene MAC25 protein) and heat shock proteins etc. Interestingly, we found high redundancy of the EST homologues of anti-lipoplysaccharide factor (16 clones, 39% of immune genes) and 11.5 kDa antibacterial protein

Table 2. *V. harveyi* infected *P.monodon* ESTs homologues classified into the defense and homeostasis group

Accession no.	Putative identification	Closest species	Database accession	Probability	Matched (%)	Sequence Length (bp)	Redundancy
BI784440	P lysozyme structural	<i>Mus musculus</i>	NPO38618	2.00E-26	67	541	2
BI784441	Penaeidine-3k precursor	<i>Litopenaeus setiferus</i>	AAK83450	9.00E-11	54	647	1
BI784442	Fc fragment of IgE	Homosapiens	NP001993	5.00E-07	67	533	-
BI784443, BI784444, BI784445, BI784446, BI784447	11.5 kDa antibacterial protein	<i>Carcinus maenas</i>	CAB51030	2.00E-10	66	687	8
BI784448, BI784449, BI784450, BI784451	antilipopolysaccharide factor	Atlantic horseshoe crab	A23931	7.00E-19	64	512	16
BI784452	Heat shock-like protein (HSP70)	<i>Ceratitis capitata</i>	AAC23392	3.00E-13	91	403	-
BI784453	proteinase inhibitor-signal crayfish	<i>Pacifastacus leniusculus</i>	S45677	6.00E-22	60	503	-
BI784454	Cytosolic manganese superoxidase Dismutase precursor	<i>Callinectes sapidus</i>	AAF74771	E-129	86	930	-
BI784455	prophenoloxidase activating factor	<i>Holotrichia diomphalia</i>	CAC12665	7.00E-18	70	550	-
BI784456	Gene MAC25 protein (Kazal proteinase inhibitor homology)	human	I52825	9.00E-10	53	405	3
BI784457	Whey acidic protein (putative Protease inhibitor)	<i>Trichosurus vulpecula</i>	AAK69407	5.00E-06	48	712	-
BI784458	Heat shock cognate 70 kD protein	<i>Oncorhynchus mykiss</i>	P0818	9.00E-95	76	772	-
BI784459	Penaeidin-3c precursor	<i>Litopenaeus vannamei</i>	P81060	1.00E-18	66	412	-
BI784460	Peptidyl-prolyl cis-trans isomerase 5	<i>Caenorhabditis elegans</i>	P52013	1.00E-60	78	570	3

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PMantiLPS-1   M RVLVSPFIMALSLIALM-----PRCQGQGVQDLLPALVEKIAG-----LWHSDEVEF
PMantiLPS-2   M RVLVSPFIMALSLIALM-----PRCQGQGVQDLLPALVEKIAG-----LWHSDEVEF
PMantiLPS-3   -M RVS---VLVSLVLVSLVALFAPQCAQGWENVAVAVASKIVG-----LWRNEKTEL
PMantiLPS-4   -M YLSSYLISLTVTVLVKYHSSPSSLFLCHFLLI PRLHFSNLFVRSPPTRLWRNEKTEL
T. tridentatus -----EGGIWTQLALALVKNLAT-----LWQSGDFQF
                :        :
                :        :
                :        :
                :        :
                :        :

PMantiLPS-1   L SHSCRYSRPSFYRWELVFNGRMCEPGWAFPTGRCE-----
PMantiLPS-2   L SHSCRYSRPSFYRWELVFNGRMCEPGWAFPTGRSRTSPSGAIEHATRDFVQKALQSN
PMantiLPS-3   LGHECKFTVKPKYLKRQVYKGRMCEPGWTAIRGEASTRSQSGVAGTKAKDFVRKAFQKG
PMantiLPS-4   LGHECKFTVKPKYLKRQVYKGRMCEPGWTAIRGEASTRSQSGVAGTKAKDFVRKAFQKG
T. tridentatus LGHECHYRVPNTVKRLKWKYKGFWCPSWTSITGRATKSSRSGAVEHSVRDFVSAKSSG
                ***::: . . . * : : : ** * . : . .
    
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Fig. 1 Sequence alignment of deduced amino acids of PMantiLPS-1, PMantiLPS-2 and PMantiLPS-3 in *P. monodon* with anti-LPS factors from *T. tridentatus*.²⁵⁾

(8 clones, 19.5% of immune genes) indicating that they are abundant molecules in haemocytes of the infected shrimp. In contrast, Gross et al. 2001,¹⁷⁾ found that the transcripts of penaeidins were predominate in haemocytes of *L. vannamei* and *L. setiferus*. Additionally, we examined the random sequences from the haemocyte cDNA library obtained from apparently healthy *P. monodon* and found the anti-LPS factor ESTs at lower frequency (5 clones from 615 cDNA sequenced). The increase in anti-LPS factor transcripts found in this infected cDNA library suggesting that this protein may be one of the major defense molecule acting against Gram-negative bacteria in *P. monodon*.

Anti-LPS factor, originally identified from the horseshoe crabs, *Limulus polyphemus* and *Tachypleus tridentatus*, binds and neutralizes lipopolysaccharide, which is the major component of the cell surface of Gram-negative bacteria^{22,23)}. Analysis of nucleotide sequences of 16 transcripts of antilipo-polysaccharide factors in this cDNA library showed sequence

variability suggesting that a least 4 types of the protein exist in the *P. monodon* haemocytes (accession no. BI784448-BI784451). They contain open reading frames of 252, 360, 369 and 396 bp coding for proteins of 84, 120, 123 and 132 amino acids, respectively. The deduced amino acids of these anti-LPS factors showed 57-65 % homology with those of *Tachypleus tridentatus* and *L. polyhemus* (Figure. 1). All of the ESTs of *P. monodon* anti-LPS factors showed 2 conserved cysteine residues and a highly conserved cluster of positive charged residues within the disulfide loop. This region is important for LPS binding.^{24,25)}

A second most common ESTs clones of antimicrobial peptides was the 11.5 kDa antibacterial protein. This protein was previously identified in the shore crab, *Carcinus maenas*.²⁶⁾ It acts only against Gram-positive marine bacteria. From the deduced amino acids of these ESTs, at least 5 types of this antibacterial protein were identified in *P. monodon* haemocytes. Two full-length clones were identified and they contain open reading frames of 321 and 402 bp encoding 107 and 134 amino acids, respectively. Comparing the deduced amino acids of these proteins with that of *C. maenas*, showed 45% and 46 % homology. Moreover, they contain all 12 conserved cysteine residues found in the 11.5 kDa anti-bacterial protein of *C. maenas* (Figure 2).

Our results revealed that haemocytes of *P.monodon* infected with the Gram-negative *V. harveyi* highly expressed antimicrobial proteins suggesting that the antimicrobial activity is one of the major defense mechanisms against invading bacteria.

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PM11.5kDa-1 -----VEVDPEVDPEASQSVTAPPASCRRWCTPEN
PM11.5kDa-2 VVSAHGGRGARPGGFPAGVPGGFGVGFGEFPAHLGGFLSVTAPPATCRRWCRTPEDA
C.maenas -----NKDKYWKDNLGL
*:.:.
PM11.5kDa-1 FYCCESRYEPAFVGTKILL-CPKVRDTCPPVRFVLAVEQVPCSSDYKCGGLDKCCFDRC
PM11.5kDa-2 VYCCESRYEPAFVGTKPLD-CPVRVDTCPVRFVGLA-PVTCSSDLKCGGLDKCCFDRC
C.maenas NYCCGGGVTYPPFTKRHLGRCPAVRDTCTGVRTQLPT---YCPHDGACFRSKCCYDTC
*:.:.
PM11.5kDa-1 LGQHVCKPFSFYEFFA
PM11.5kDa-2 LKEHVCKPFSFYSHFA
C.maenas LKHHVCKT-AEYPPY--
*:.:.

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Fig. 2. Sequence alignment of deduced amino acids of PM11.5kD-1, and PM11.5kD-2 in *P. monodon* with 11.5 kDa antibacterial protein from *C. maenas*.²⁶⁾

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Biography

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