Proteome analysis of abundant proteins in early Atlantic cod (*Gadus morhua*) larvae

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ABSTRACT

The production of good quality larvae is a challenge in marine fish hatcheries. In this study, the proteome profile of Atlantic cod (*Gadus morhua*) was analyzed using 2-DE, MALDI TOF MS and LC-MS/MS in order to determine its protein composition. Out of 109 abundant spots analyzed with MS 77 proteins were identified. The identified proteins were classified into 5 groups: structural proteins (56%), cytosolic proteins (29%), mitochondrial proteins (7%), secreted and extracellular proteins (7%) and nuclear proteins (1%). This study is the first step in a future construction of an Atlantic cod protein database that will be a valuable resource for molecular analysis of marine fish larval development.

Keywords: Atlantic cod larvae, proteome, 2-DE, MALDI-TOF MS, LC-MS/MS

YFIRLIT

Greining á helstu próteinum í próteinmengi þorsklirfa á frumfóðrunarstigi

Afkoma og gæði er einn megin flöskuhálsinn við framleiðslu sjávarfiska í eldi. Í þessari rannsókn var próteinmengi þorsklirfa aðgreint á tvívíðum rafdráttargeljum. Um 450 próteindeplar, með mólmassa á bilinu 6-100 kDa og jafnhleðslupunkt (pI) á bilinu pH 4-7, voru greindir. Rúmlega 100 próteindeplar voru skornir út úr rafdráttargeljum til nánari skoðunar. Um 70% þeirra voru kennigreindir með massagreiningum (MALDI-TOF MS og LC-MS/MS) og upplýsingum úr prótein gagnabönkum (Swiss-Prot og NCBInr). Stór hluti próteindeplanna voru úr vöðva og frumugrind eins og búast mátti við þar sem greiningin var gerð á heilum lirfum. Niðurstöður rannsóknarinnar eru fyrsta skrefið í uppbyggingu á gagnabanka fyrir próteinmengi þorsklirfa sem mun þjóna mikilvægum tilgangi í rannsóknum á lirfum sjávarfiska í framtíðinni.

INTRODUCTION

The production of good quality larvae is a challenge in marine fish hatcheries. Several environmental factors can interfere with the protein expression of larvae affecting larval quality, like growth and survival rate.

Proteome analysis allows us to examine the effects of environmental and nutritional factors on larval global protein expression and post-translational modifications (PTMs) (Tyers & Mann 2003), all important information for controlling factors influencing the aptitude to continue a normal development through to adult stages.

The methodology used for proteome analysis is based on protein extraction from a whole organism or tissue. The proteins are then separated by two-dimensional gel electrophoresis (2-DE) according to their isoelectric point (pI) and molecular mass, yielding a 2-D map of the proteome (Görg et al. 2004). Depending on the gel size and pH gradient used, the 2-DE is capable of resolving thousands of proteins simultaneously (~2000 proteins routinely) and can be used to detect and quantify protein amounts of less than one ng per spot (Görg et al. 2004). Furthermore, it delivers a 2-D map of intact proteins, which can reflect changes in the protein expression level, isoforms or PTMs. For an exact protein identification, protein spots of interest (e.g. up- or down-regulated proteins) are excised from the 2-D gel, digested into fragments by specific proteases and then identified using mass spectrometry (MS) and database mining (Aebersold & Mann 2003).

Only a few proteome analysis studies on early developmental stages of fish have been published. Three of these studies have focused on the larval proteome of Atlantic cod (*Gadus morhua* Linneaaus) (Sveinsdóttir et al. 2008, 2009, Sveinsdóttir & Gudmundsdóttir 2010) while others are on the embryonic proteome of zebrafish (*Danio rerio* Hamilton) (Tay et al. 2006, Lucitt et al. 2008).

Here we report the proteome map of early cod larvae at the age of 6 days post hatch (dph) prepared by 2-DE and MS analysis. This provides a basic overview of abundant proteins in early cod larvae for designing further proteomics studies with diverse purposes, including changes in protein expression in response to different environmental factors.

MATERIALS AND METHODS

Fish larvae

Atlantic cod larvae at age 6 dph were obtained from the Marine Research Institute at Stadur near Grindavík, Iceland, a commercial production unit for cod hatchery. Details of egg incubation and larvae rearing conditions at the Marine Research Institute at Stadur have been published in Steinarsson & Björnsson (1999). At day 6 ph larval samples for proteome analysis were collected prior to morning feeding. The larvae were rinsed thoroughly with distilled water and quickly frozen in liquid nitrogen. Samples were collected from four 150 L silos.

Protein extraction

Total protein extracts were performed from pooled larval samples (10 larvae per sample) as described by Sveinsdóttir et al. (2008). Four replicates were done for each larval group to ensure reproducibility.

Analysis of fish proteins by 2-dimensional gel electrophoresis

Proteins were analyzed by 2-DE on medium-format 2-D gels (11 cm immobilized pH 4-7 gradient gel strips, IPG gel strips (BioRad, Hemel Hempstead, UK)). To determine appropriate loading for the gels, aliquots of the fish proteins were analyzed by one-dimensional (1D) SDS PAGE. Subsequent gel loading was determined based on the intensity of the stained 1D profile; typically 500 μg of protein were loaded to a 2-D gel. The 2-D gels were processed according to Sveinsdóttir et al. (2008). The resolved proteins were detected using a modified Coomassie Colloidal Blue G250 staining (Cash 1995). After staining the gels were scanned with a Personal Densitometer S1 (Molecular Dynamics, Sunnyvale, USA) and transferred as 12 bit images at a res-

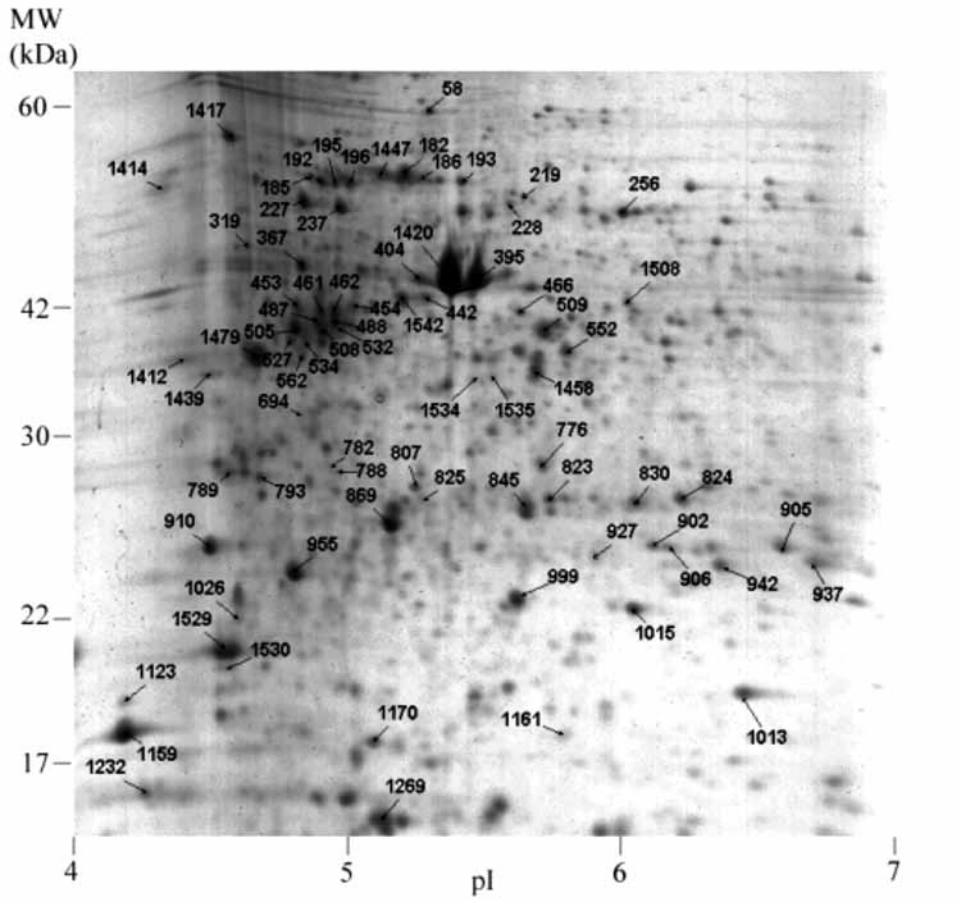

Figure 1. 2-D map of cod larval proteins. A total larval protein extract was prepared from early larvae at the age of 6 dph. The first dimension was performed by IEF on pH 4-7 lineal IPG strips, the second dimension on a 12% SDS-PAGE gels, and the proteins were visualized by Coomassie colloidal blue G250. The indicated spots were identified by MALDI-TOF MS and LC-MS/MS. The y-axis indicates the molecular weight (MW) and the x-axis shows the isoelectric point (pI).

olution of 50 µm to Progenesis PG200 2006 (NonLinear Dynamics, Newcastle upon Tyne, UK).

Image analysis of 2-D protein profiles

All image analysis was carried out using the Progenesis PG200 2006 software. Spots were detected using a combination of in-built computer routines and minimal manual editing to remove artifacts. A protein profile from L2-gel was selected as the basis for the construction of a reference gel, against which the remaining protein profiles (L1, L3 and L4) were matched using built-in software routines. Each spot within the reference protein profile was assigned a spot number, which was used in the subsequent description to refer to individual spots. Spot abundance, pI's and molecular weights were assigned to all spots. The spot volume is the sum of pixel intensities within the boundary of that spot. Following removal of background, the spot volume was normalized to the total protein detected for each protein profile. The normalized spot volume is referred to as abundance. The pI's of the proteins were calculated from the pH gradient of the IPG gel using the manufacturer's data. Molecular weights were determined

by co-electrophoresis with proteins of known molecular masses.

Average gel for the larval proteome was derived from four replicates (L1-L4); spots were excluded if they were absent from one or more of the replicate larval proteomes. Spots in the average gels with a normalized spot volume ≥ 0.15 were assigned as highly abundant spots.

Protein identification by peptide mass fingerprinting (PMF) and peptide fragment fingerprinting (PFF)

Proteins of interest (spots with normalized spot volume ≥ 0.15) were excised from the stained gels and subjected to in-gel digestion as previously described in Sveinsdóttir et al. (2008). In short, a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, CA, USA) was used to analyze the tryptic digested peptides. The analysis was done in the reflection-delayed extraction mode. The initial calibration of the spectra was carried out using tryptic-digestion autolysis peptides.

Protein identification was performed using the Mascot search engine (http://www.matrixscience.com). NCBInr (http://www.ncbi.nlm.nih.gov) and Swiss-Prot (http://www.expasy.org/sprot/) databases were selected as the primary databases to be searched. Mascot search parameters were as follows: maximum allowed error of peptide mass 50-150 ppm, maximal number of missed cleavage was 1, carboxiamidomethylation of cysteine and oxidation of methionine allowed. The searches were taxonomically restricted to *Actinopterygii* (ray-finned fishes).

Protein spots not unambiguously identified by peptide mass fingerprinting (PMF) were further examined by LC-MS/MS fragmentation (Sveinsdóttir & Gudmundsdóttir 2010).

For both MS methods, the presence of 2 matching peptides was the minimal requirement for an identity assignment. The automatically identified proteins were checked individually.

RESULTS AND DISCUSSION

Analysis of the larval 2-D protein profile

The number of protein spots in the cod larval proteome, analyzed on the 2-DE gels after editing, was on average 450.75 ± 28.61 (mean ± SEM). A total of 450 proteins is only a small fraction of all the proteins expressed in eukaryotic cells. The proteins detected were readily dissolved and expressed at a high level in the cell with a molecular weight between 7–100 kDa and pI's between pH 4 and 7. The major problem concerning proteome analysis from an un-fractionated sample, as in the current study, lies in the highly dynamic range of protein abundance, as well as the diversity of proteins with respect to their molecular weight, pI and solubility (Görg et al. 2004). Hence, 2-DE analysis of unfractionated tissue samples is constrained to analysis of the most abundant proteins.

Identification of protein spots by mass spectrometry

A total of 109 protein spots were selected for identification by PMF or PPF based on their abundance. The localizations of these spots on the larval 2-D protein profile are shown in Figure 1. A total of 77 protein spots (identification rate of 71%) were identified. The results of the MALDI-TOF MS and LC-MS/MS analyses are summarized in Table 1. Thirty-two protein spots could not be identified. Attaining a high identification rate is problematic in fish proteomics due to the relative paucity of available protein sequence data for these animals. However, it is of great importance to increase knowledge in this field, especially for species of commercial importance like the Atlantic cod. Currently, (March, 2011) there are 2,086 nucleotide sequences and 2,217 protein sequences identified from the Atlantic cod (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/). This level of identification is also reflected in the Mascot search results as only 17 proteins (22%) of the identified cod larval proteins match to protein sequences originating from cod.

Among the abundant identified proteins in

early cod larvae, 50 proteins were expressed as multiple spots, demonstrating that they are isoforms. This is in accordance with results in other proteomics studies (Sveinsdóttir et al. 2008, 2009, Sveinsdóttir & Gudmundsdóttir 2010, Ziv et al. 2008). Detection of isoforms has been considered to be one of the greatest strengths of proteome analysis based on 2-DE. Cellular response mechanisms are largely limited to post-transcriptional and post-translational processes resulting in differential expression of protein isoforms. Thus, proteome analysis is an important addition to studies based on mRNA detection to increase understanding of the cellular responses to environmental stimuli.

The identified cod larval proteins were divided into 5 groups involving structural proteins (44/77 - 56%), cytosolic proteins (22/77 - 29%), mitochondrial proteins (5/77 - 7%), secreted and extracellular proteins (5/77 - 7%), and nuclear proteins (1/77 – 1%).

Structural proteins

Actins were the most predominant proteins among the cytoskeletal proteins, accounting for 25% of the identified proteins of the early larval proteome. Ten protein spots were identified as muscle α-actin, of which 7 originated from Atlantic cod. Most of the larval proteins up-regulated upon probiotic bacteria treatment were found to be muscle α-actin, indicating an enhanced development of treated larvae (Sveinsdóttir et al. 2009).

Other predominant protein spots corresponding to structural proteins were myosin, tubulins and keratins. Nine protein spots were identified as type II keratin originating from gilthead sea bream (*Sparus aurata*) and one spot as cytokeratin type I from zebrafish enveloping layer. Proteome analysis of two age groups of cod larvae showed that type II keratin is more predominant in larvae at the beginning of first feeding and type I is found in higher amounts in older larvae (Sveinsdóttir et al. 2008), indicating a developmental stage-specific expression of these proteins.

Cytosolic proteins

Another important group of larval proteins identified in the present study were cytosolic proteins involved in stress response, energy production and metabolism. Two protein spots were identified as the 70-kdalton (kDa) heat shock cognate protein (HSC70). HSC70 is expressed in high amounts in fish skeletal muscle where it has been related to muscle growth (Park et al. 2001). A proteome analysis of cod larvae revealed a developmental stage-specific expression of HSC70 (Sveinsdóttir et al. 2008). Two protein spots were found to be most similar to the mitogen-activated protein kinase p38 (MAPK p38), a protein that is related to immune response. A down-regulation of MAPK p38 was observed in early cod larvae after treatment with probiotic bacteria (Sveinsdóttir et al. 2009). Creatine kinase (CK) and the vacuolar-type of H^+-transporting ATPase (V-type H^+-ATPase) are cytosolic enzymes involved in energy metabolism. Expression of these enzymes was affected by treatment with fish hydrolysates during the first feeding stage of cod larvae (Sveinsdóttir & Gudmundsdóttir 2010), indicating a role in the response to this environmental stimulus. In the same study a down-regulation of tyrosine 3-monooxygenase/tryptophan activation protein β-polypeptide (YWHAß) was observed, a protein that has only been detected in early cod larvae (Sveinsdóttir et al. 2008). Other identified cytosolic proteins in the larval proteome included oxygen-carrying proteins such as cytoglobin and myoglobin. Parvalbumin, a calcium binding albumin protein having a role in muscle relaxation, prohibitin, an anti-proliferative protein that inhibits DNA synthesis and Histamine N-methyltransferase (HMT) that can be related to immune response as it plays a crucial role in the inactivation of histamine (Tahara et al. 2000), were also found. Enolase and glycerol-3-phosphatase dehydrogenase (GPDH) were among identified proteins of the larval proteome. Enolase is a part of the glycolytic pathway and it is considered to be a useful marker in the study of differentiation and pathological alteration. It has been shown that

Table 1. Proteins identified in early Atlantic cod larvae using MALDI-TOF MS and LC-MS/MS.

Spot	Accession			MW/pI[1]	MW/pI		SC	
no.	no.	Protein identification	Species	Theoretical	Observed	PM[2]	(%)[3]	MS method
		Cytoskeletal proteins						
505	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	38.7/4.95	14	27	LC-MS/MS
462	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	40.4/5.10	5	16	LC-MS/MS
508	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	39.2/5.04	9	28	MALDI-TOF
488	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	39.3/5.18	9	28	MALDI-TOF
487	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	40.0/5.12	6	15	LC-MS/MS
453	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	40.0/5.12	6	15	LC-MS/MS
534	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	37.9/5.00	12	25	LC-MS/MS
562	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	37.7/5.07	8	21	LC-MS/MS
694	Q4QY72	Type II keratin	*Sparus aurata*	38.6/4.89	32.1/5.04	10	22	LC-MS/MS
532	Q6P0E4	Type I cytokeratin	*Danio rerio*	46.7/5.13	37.5/5.13	7	7	LC/MS-MS
193	P30436	Tubulin α chain	*Oncorhynchus keta*	50.0/4.92	52.3/5.51	12	29	MALDI-TOF
1447	P30436	Tubulin α chain	*Oncorhynchus keta*	50.0/4.92	53.8/5.25	16	35	MALDI-TOF
182	Q6P5N0	Tubulin, α 8 like 2	*Danio rerio*	50.8/4.97	54.2/5.33	17	40	LC/MS-MS
195	Q9PUG4	β-2 tubulin	*Gadus morhua*	50.0/4.71	52.9/5.19	11	35	LC-MS/MS
186	Q9PUG4	β -2 tubulin	*Gadus morhua*	50.0/4.71	52.5/5.38	35	58	LC-MS/MS
1414	Q9PUG4	β -2 tubulin	*Gadus morhua*	50.0/4.71	51.5/4.42	13	23	MALDI-TOF
196	Q9PUG4	β -2 tubulin	*Gadus morhua*	50.0/4.71	52.5/5.15	13	23	MALDI-TOF
185	Q9PUG5	β -2 tubulin	*Gadus morhua*	50.0/4.71	52.5/5.00	38	60	LC-MS/MS
192	Q9PUG4	β -2 tubulin	*Gadus morhua*	50.0/4.71	51.6/5.05	18	43	MALDI-TOF
1458	Q91037	Actin (Fragment)	*Gadus morhua*	37.3/5.25	36.3/5.81	6	19	MALDI-TOF
845	B4ZYS5	β -actin (Fragment)	*Oreochromis aureus*	15.7/6.92	28.2/5.77	9	44	MALDI-TOF
1542	Q802U0	β actin	*Dicentrarchus labrax*	42.1/5.29	41.9/5.32	7	27	MALDI-TOF
454	P53486	Actin, cytoplasmic	*Fugu rubripes*	42.0/5.30	40.8/5.16	12	37	LC/MS-MS
442	P53486	Actin, cytoplasmic	*Fugu rubripes*	42.0/5.30	40.9/5.30	20	42	LC-MS/MS
782	P53486	Actin, cytoplasmic	*Fugu rubripes*	42.0/5.30	29.9/5.17	2	5	LC-MS/MS
1534	P68142	Actin, cytoplasmic	*Fugu rubripes*	42.1/5.30	34.7/5.69	9	29	MALDI-TOF
1161	Q0EDA7	β – actin	*Pagrus major*	14.0/5.81	17.5/6.00	2	21	LC-MS/MS
395	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	43.6/5.57	13	28	MALDI-TOF
509	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	39.5/5.83	11	31	MALDI-TOF
404	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	43.6/5.38	14	38	MALDI-TOF
776	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	29.9/5.91	18	46	LC-MS/MS
825	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	28.2/5.39	10	33	LC-MS/MS
927	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	25.1/6.09	14	30	LC-MS/MS
1535	Q78AY8	Muscle α-actin	*Gadus morhua*	42.3/5.23	34.2/5.79	20	39	LC-MS/MS
461	P49056	Muscle α –actin	*Carassius auratus*	42.3/5.23	40.1/5.12	29	60	LC/MS-MS
1420	Q9PTJ5	Muscle α –actin	*Sparus aurata*	42.2/5.28	43.6/5.57	9	27	MALDI-TOF
1508	P53482	Muscle B, α -actin	*Fugu rubripes*	42.3/5.22	42.9/6.11	13	41	MALDI-TOF
1479	P84335	Tropomyosin-1	*Liza aurata*	32.8/4.69	36.5/4.80	9	26	MALDI-TOF
1529	O93409	Myosin, light chain	*Danio rerio*	19.0/4.68	20.6/4.68	9	50	MALDI-TOF
1159	Q9IB38	Myosin light chain	*Thunnus thynnus*	18.5/4.38	18.6/4.35	6	26	MALDI-TOF
955	Q9IB19	Myosin light chain	*Theragra chalcogramma*	21.4/4.70	24.8/4.94	10	42	MALDI-TOF
1026	Q9IB19	Myosin light chain	*Theragra chalcogramma*	21.4/4.70	21.2/4.78	5	34	LC-MS/MS
905	B1MUD4	Crystallin β-A2	*Dissostichus mawsoni*	23.7/6.00	26.6/6.70	11	30	LC-MS/MS
1013	Q6DG28	Crystallin β-B1	*Danio rerio*	27.3/6.44	19.7/6.56	2	10	LC-MS/MS
		Cytosolic proteins						
256	Q6PC12	Enolase	*Danio rerio*	47.4/6.20	49.8/6.10	12	25	MALDI-TOF
466	Q6DC37	HMT	*Danio rerio*	33.9/5.14	40.7/6.74	5	22	MALDI-TOF
552	Q6GUQ0	GDPH	*Gadus morhua*	38.7/5.47	38.2/5.91	10	37	LC-MS/MS
1439	Q6P102	YWHAß	*Danio rerio*	28.0/4.71	35.5/4.60	2	10	LC-MS/MS

Spot no.	Accession no.	Protein identification	Species	MW/pI[1] Theoretical	MW/pI Observed	PM[2]	SC (%)[3]	MS method
1412	Q6P102	YWHAß	*Danio rerio*	27.7/4.71	35.9/4.53	4	11	LC-MS/MS
367	Q8UWM8	HSC70	*Xiphophorus maculatus*	70.9/5.44	43.7/4.97	11	18	MALDI-TOF
58	P08108	HSC70	*Oncorhynchus mykiss*	71.3/5.24	61.5/5.41	3	22	LC-MS/MS
942	A7XA06	Creatine kinase	*Gadus morhua*	27.4/6.63	24.6/6.51	12	27	LC-MS/MS
902	A7X9Z6	Creatine kinase	*Hippoglossus hippoglossus*	27.5/6.86	25.6/6.33	4	10	LC-MS/MS
906	Q7T1J2	Creatine kinase	*Chaenocephalus aceratus*	42.9/6.40	26.6/6.30	3	8	LC-MS/MS
1170	Q9DGE1	MAPK p38	*Danio rerio*	40.1/6.36	17.7/5.23	5	18	MALDI-TOF
1123	Q9DGE1	MAPK p38	*Danio rerio*	40.1/6.36	19.1/4.35	5	18	MALDI-TOF
824	Q7ZV03	Vacuolar protein	*Danio rerio*	38.7/6.20	29.0/6.33	5	23	MALDI-TOF
869	Q5EAQ1	MIF4G domain-containing protein	*Danio rerio*	26.0/5.08	28.5/5.19	2	28	MALDI-TOF
219	Q9PUK7	V-type H^+-ATPase	*Anguilla anguilla*	56.2/5.33	52.8/5.84	8	21	MALDI-TOF
228	Q9PUK7	V-type H^+-ATPase	*Anguilla anguilla*	56.2/5.33	51.7/5.78	3	8	LC-MS/MS
823	Q575S9	Cytoglobin-2 OS	*Oryzias latipes*	20.5/5.22	28.7/5.86	5	39	MALDI-TOF
1417	O73813	40S ribosomal	*Oryzias latipes*	30.2/9.74	54.7/4.70	9	30	MALDI-TOF
807	Q7T1D8	Prohibitin	*Danio rerio*	29.7/5.28	28.8/5.37	5	18	LC-MS/MS
1232	P02622	Parvalbumin β	*Gadus callarias*	12.2/4.37	15.9/4.43	2	24	MALDI-TOF
910	P59747	Parvalbumin β	*Scomber japonicus*	11.7/5.15	25.9/4.61	4	38	MALDI-TOF
1269	Q9DGJ0	Myoglobin	*Sarda chiliensis*	15.8/9.13	17.3/5.16	6	33	MALDI-TOF
		Mitochondrial proteins						
227	Q9PTY0	ATP synthase	*Cyprinus carpio*	55.3/5.05	49.4/5.00	18	39	MALDI-TOF
237	Q9PTY0	ATP synthase	*Cyprinus carpio*	55.3/5.05	49.7/5.12	17	38	MALDI-TOF
319	Q9PTY0	ATP synthase	*Cyprinus carpio*	55.3/5.05	45.3/4.82	27	46	LC-MS/MS
999	Q501S4	Methyltransferase	*Danio rerio*	28.8/7.59	23.1/5.74	6	20	MALDI-TOF
830	Q58EJ9	MOSC domain-containing protein	*Danio rerio*	37.1/6.28	28.7/6.16	6	17	MALDI-TOF
		Secreted and extracellular proteins						
1015	P00761	Serine protease	*Danio rerio*	25.1/7.00	23.7/6.16	2	8	LC-MS/MS
793	Q7T1G9	Transferrin variant A	*Carassius cuvieri*	76.2/5.89	29.3/4.81	11	16	MALDI-TOF
788	P84610	Haemoglobin subunit β-[1]	*Gadus morhua*	16.6/6.58	29.6/5.09	5	23	MALDI-TOF
1530	P26774	Somatotropin-[2]	*Acipenser guldenstadti*	22.0/7.00	20.0/4.67	3	16	MALDI-TOF
527	P45641	Somatolactin precursor	*Hippoglossus hippoglossus*	26.9/5.29	37.7/4.93	5	23	MALDI-TOF
		Nuclear proteins						
789	Q9I879	ARNT	*Danio rerio*	69.6/6.35	29.0/4.68	8	13	MALDI-TOF

[1] MW/pI: Molecular weight and isoelectric point
[2] Peptide matched.
[3] Sequence coverage

protein expression and activity of enolase is related to regeneration and development of the muscle (Merkulova et al. 2000). GPDH is also a part of the glycolytic pathway and it has been described as a marker for the glycolytic potential (White et al. 2000).

Other proteins

About 15% of the cod larval proteins identified appear to originate from the mitochondria, the nucleus or the secretion pathway. Several protein spots were identified as mitochondrial ATP synthase. There is an important relationship between hypoxia tolerance and ATP con-

centration in tissue; therefore the ability to produce ATP in a stressful environment is fundamental to stress tolerance (Ishibashi et al. 2007). Other proteins identified were the aryl hydrocarbon receptor nuclear translocator (ARNT), transferrin and hemoglobin, which all have been shown to play a role during development and in response to environmental stimuli of fish (Magnadóttir et al 2004, Zinkernagel et al. 2007, Brunt et al. 2008, Caipang et al. 2008).

In conclusion, this study represents the 2-D protein profile of first feeding Atlantic cod larvae (6 dph) and the identification of 77 abundant larval proteins. This is the first step toward a future construction of an Atlantic cod protein database that will be a valuable resource for molecular analysis of marine fish larval development.

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