Research Article

Proteomic Profiling of Freeze- and Spray-Dried Water Extracts of Snakehead Fish (*Channa striatus*): In Search of Biomolecules for Wound Healing Properties

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Abstract | *Channa striatus* is a carnivorous freshwater fish that is commonly consumed among Malaysians. The fish is known to contain compound(s) that can accelerate the wound healing process in humans, but the attributes of these compound(s) are yet to be clarified. In the current study, we have performed a thorough proteomic profiling of spray -dried and freeze-dried *C. striatus* water extracts using high-sensitivity liquid chromatography tandem mass spectrometry. Other than the analysis of whole sample, both samples were also fractionated in order to maximise protein detection. About 137 and 194 proteins were identified in spray dried and freeze dried samples, respectively. Actin, myosin, tropomyosin, calcium ion-related protein and collagen are among the proteins that have been identified, and which are suspected to be involved in the wound healing process. A high number of uncharacterised proteins were also detected, which suggested that there are still many fish proteins with unknown functions in *C. striatus*. In the future, the identified proteins can be isolated and further studies are required for a better understanding on the wound healing property of *C. striatus*.

Keywords | *Channa striatus*, Fish, Mass spectrometry, Proteomics, Wound healing

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INTRODUCTION

Channa striatus, is a carnivorous freshwater fish that is commonly found in Malaysia and is commonly known by the locals as "*Haruan*" in the Malay language. It is the most widely introduced snakehead fish under the family of *Channidae* (Courtenay and Williams, 2004). Other countries where we can encounter such species include the subtropical countries such as China, Taiwan, Thailand, Philippines, Indonesia and India (Mohsin and Ambak, 1983). Although currently such snakehead fish is cultured commercially in Thailand, Philippines and India (Gam et al., 2006), it was not given priority in the local farming industry as the locals believe it to be a pest (Jais, 2007).

C. striatus has long been a recommended part for the patients' diet that have just gone through surgery or delivery as the locals believe that it helps in the healing process (Jais et al., 1994). *C. striatus* flesh is thought to contain medicinal properties and its consumption helps in the recovery from serious illness (Wee, 1982). Such theory has been supported by Jais et al. (1994), who stated that *C. striatus* contains essential amino acids for wound healing. Jais et al. (1994) also showed a high amount of arachidonic acid in *C. striatus*. Arachidonic acid is the precursor of thromboxane and prostacyclin that is responsible for blood clotting (Nelson et al., 1997; Silver et al., 1973). In previous studies, a *C. striatus* extract has been applied on wounded mice to determine the tensile strength of the healed wound (Baie and Sheikh, 2000a, 2000b). They reported that the high content of arachidonic acid, glycine and polyunsaturated fatty acid may have contributed to the wound healing process.

The pharmaceutical products of *C. striatus* that are currently available in the market are usually in capsule form.

C. striatus capsules are prepared by either freeze-drying or spray- drying the whole fish including the water extract. The water extract of the fish actually resembles the traditional preparation of the fish for consumption which is the soup. Both the freeze-drying (Mellor, 1978) and spray-drying (Masters, 1979) methods have their own strengths and weaknesses, which can give impacts on the protein contents retained in the end products. Both of the methods have always been compared using different samples (Anwar and Kunz, 2011; Chen et al., 2012; Maa et al., 1999) in order to identify the better option for each sample respectively. However, until currently both the freeze-dried and spray-dried samples of *C. striatus* have never been compared. In this study, we have compared the proteomes detected for both samples.

With the advancement of technology, proteins available in the fish can be now be better profiled (Doherty et al., 2012; Martyniuk and Denslow, 2012). Fish are rich in proteins that are essential and beneficial to human health. A better understanding of the interaction between the proteins in the *C. striatus* and the wound can be established through the profiling of those proteins. In addition, the proteins responsible for the wound healing effect can be isolated for medicinal and clinical purposes once identified. Despite having a preliminary protein profiling being conducted previously on the flesh of *C. striatus* (Gam et al., 2006), there were still limitations on the profiling as the equipment used in the past were not as sensitive.

In this study, we have compared the protein concentration and performed proteome profiling for both freeze-dried and spray-dried water extracts from *C. striatus*. Other than analysing the whole sample, we have also analysed fractionated samples to complement the result. The aim was to profile as many proteins as possible and postulate those that might be involved in the wound healing property of *C. striatus*. Such findings would give us an insight on how the consumption of *C. striatus* could contribute to the wound healing mechanism.

MATERIALS AND METHODS

Protein Extraction from *C. striatus* Samples

The *C. striatus* freeze- and spray-dried samples were provided by the School of Pharmaceutical Sciences, Universiti Sains Malaysia. The *C. striatus* originated from Kedah, Peninsular Malaysia. Protein extraction on both samples was performed in replicates according to the method reported by Gam et al. (2006) with minor adjustments. 15mg of freeze-dried and spray-dried *C. striatus* sample were added into 1 mL of 40 mMTris-HCl (pH 8.8) extraction buffer (Bio-Rad Laboratories, CA, USA) respectively and waited for 20 minutes with occasional vortex. Sample mixtures were then centrifuged at 12,000 x g for 30 minutes. Supernatants were recovered and kept in -35°C for subsequent analysis.

Total Protein Quantification using Bradford Assay

Total protein quantification for the sample of *C. striatus* was carried out according to Bradford (1976). 5µL of supernatant collected during the protein extraction was mixed with 250µL of Bradford reagent in a 96 well plate. The solution was then incubated for 15 minutes in room temperature. The absorbance was then measured at 595 nm. A standard curve was constructed using the bovine serum albumin (BSA). The standard curve plotted ranged from 0.0-1.4 mg/mL. The total protein concentration in each sample was determined by comparing the absorbance value obtained for the sample against the standard curve.

Protein Fractionation using Gelfree 8100 Fractionation System

The procedure was carried out on Gelfree 8100 fractionation system (Expedeon, CA, USA) according to the article by Witkowski and Harkins (2009). 200 µg of protein samples were loaded to both 8% Tris-acetate cartridge and 10% Tris-acetate cartridge. Twelve fractions were collected during the procedure, respectively. In order to confirm that the separation was successful, 10 µl of each fraction was analysed using SDS-PAGE (12.5% gel) (Bio-Rad Laboratories, CA, USA) and stained with Coomassie blue. The remaining fractions were then concentrated using a concentrator to remove the sample buffer.

Protein Digestion using Trypsin

The protein samples were re-suspended in 100µL of 6 M urea, 100mMTris buffer at 10mg/mL. The digestion method was carried out according to Kinter and Sherman (2005). Briefly, 200 mM DTT was added to each sample and kept in room temperature for 1 hour. Later, 200 mM of iodoacetamide was added and incubated in room temperature for 1 hour. 20 µL of 200 mM DTT was added next to consume any unreacted iodoacetamide. Concentration of urea in the sample was then reduced by adding 775µL of water. 20µg of trypsin (Promega, WI, USA) in solution is added to each sample and incubated overnight at 37 °C for digestion purpose. The digestion was stopped the next day by adjusting the pH of the buffer to pH <6.

LC-MS/MS Analysis

Prior to LC-MS/MS analysis, each of the peptide sample were mixed with 100ul of 0.1% formic acid in deionized water and filtered using the 0.45um regenerated cellulose (RC) membrane syringe filter (Sartorius AG, Goettingen, Germany). Analysis was performed using LTQ-Orbitrap Velos Pro mass spectrometer coupled with Easy-nLC II nano liquid chromatography system. Easy column C18 (10cm, 0.75mm i.d., 3µm; Thermo Scientific, San Jose, CA,

USA) was used as the analytical column, whereas Easy column C18 (2cm, 0.1mm i.d., 5µm; Thermo Scientific, San Jose, CA, USA) was used as the pre-column. The pre-column was equilibrated at a flow rate of 3µL/min for 15µL and analytical column was equilibrated at a flow rate of 0.3µL/min for 4µL. 5µL of samples were injected and chromatographically separated at a flow rate of 0.3µL/min. Running buffers used were: (A) 0.1% formic acid in deionized water, and (B) 0.1% formic acid in acetonitrile. Samples were eluted using the gradient 5% to 100% of buffer B in 80 minutes. The eluent was sprayed into the mass spectrometer at 2.1 kV (source voltage) and capillary temperature of 220°C was used. Peptides were detected by full scan mass analysis from m/z 300-2,000 at resolving power of 60,000 (at m/z 400, FWHM; 1-s acquisition), with data-dependent MS/MS analyses (ITMS) triggered by the 8 most abundant ions from the parent mass list of predicted peptides, with rejection of singly or unassigned charge state. Collision induced dissociation (CID) was applied as the fragmentation technique with a collision energy of 35. Each sample was analysed twice.

Protein and Peptide Identification by De Novo Sequencing

PEAKS Studio Version 7 (Bioinformatics Solution, Waterloo, Canada) was used to perform *de novo* sequencing and database matching. Uniprot fish database from October 2014 was used for the database matching. Carbamidomethylation and methionine oxidation were set as fixed modifications and maximum missed cleavage was set at 2. Parent mass and precursor mass tolerance were set at 0.1 Da. False detection rate (FDR) <0.1% and significant score (−10lgP) for protein >30 were used for protein acceptance. Maximum variable post-translational modification was set at 4.

RESULTS AND DISCUSSION

Total Protein Concentration

The total protein concentration varies for each *C. striatus* sample. The average result from four replicates is shown in Table 1. As presented, the total protein concentration in the spray-dried sample is slightly lower than the freeze-dried sample. This might be due to the high temperature introduced during the spray-drying process, which has caused denaturation on certain proteins to be available in the sample. Joshi et al. (2011) has mentioned that different drying methods have different capabilities of denaturing proteins by modifying the protein structures. Figure 1, which is a SDS-PAGE image, showed the application of Gelfree fraction system able to separate proteins in the *C. striatus* samples. The fractionated proteins allow the mass spectrometry to detect low abundance proteins available in the samples.

Table 1: Average total protein concentration in *C. striatus* free-dried and spray-dried samples

Sample	Average total protein concentration (mg/ml)
Freeze-dried (FD) water extracts	1.242
Spray-dried (SD) water extracts	1.160

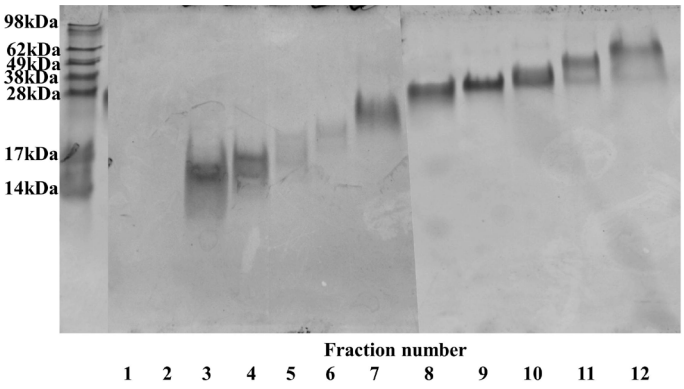

Figure 1: Image of fractionated proteins after SDS-PAGE gel
12 fractions were collected from freeze-dried and spray-dried samples, respectively.

Many proteins are heat-sensitive and thus spray-drying has been less favourable in the pharmaceutical industry. During the spray-drying process, the denaturation temperature of protein is greatly affected by the water content in the sample (Maltesen and Van De Weert, 2008). Proteins are most sensitive to thermal denaturation after atomisation. Such phenomenon gives an indication that proteins face a higher risk of denaturation in spray-drying method. It explains the reason for the lower protein concentration obtained in the spray-drying sample. Therefore, the pharmaceutical industry and research work prefer the freeze-drying method (Maltesen and Van De Weert, 2008), whereas the food industry tends to select the spray-drying method.

Comparison of Proteome Profiles Obtained from Freeze- and Spray-Dried *C. striatus* Water Extracts

Protein identification by nano-LCMS/MS showed that 137 proteins and 194 proteins were identified in spray-dried and freeze-dried samples respectively. The amount of proteins shown consists of the accumulation of whole sample as well as the fractions obtained from Gelfree fractionation. The number of proteins identified is higher compared to the previous proteomic analysis by Gam et al. (2006), which only obtained 85 proteins, which suggested that the utilization of advanced Easy-nLC II nano liquid chromatography system enables more minor proteins to be detected. Such result was in line with the total protein concentration obtained, where the freeze dried sample is higher. A portion of proteins in the *C. striatus* sample that

are heat-sensitive might have been denatured during the spray drying process. 97 of the proteins identified in the freeze-dried sample were similar to the proteins identified in the earlier spray-dried sample as shown in Figure 2. The similar proteins were mainly consisted of structural proteins and uncharacterised proteins.

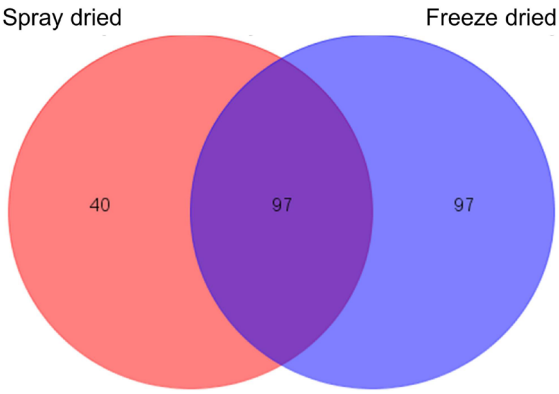

Figure 2: Venn diagram of protein numbers detected in both spray-dried (left) and freeze-dried (right) *C. striatus* water extracts

A number of 97 identical proteins were detected on both samples.

Role of Proteins in Wound Healing

In general, three different but overlapping phases can be identified in the wound healing process which is: (1) haemostasis and inflammation, (2) proliferation or granulation, and (3) maturation or remodelling (Flanagan, 2000; Singer and Clark, 1999; Witte and Barbul, 1997). Different proteins are involved and very often they are integrated into a complex mechanism. Several proteins that were identified in the *C. Striatus* could give us an insight on how it enhances the wound healing mechanism. The list of proteins detected for both samples were listed in Table 2 and Table 3. Due to the limited entries in the protein database for *C. striatus*, a large amount of proteins detected from the database were from different species other than *C. striatus*. This also showed the possibility that the proteins/peptides available in *C. striatus* share the same sequence with other fish species as well.

Actin, Myosin and Tropomyosin

Actin, myosin and tropomyosin constitute 25% and 26% of the total protein detected in freeze- and spray-dried *C. striatus* water extracts, respectively (Figure 3). According to Dominguez and Holmes (2011), actins are essential for cellular functions such as motility, the maintenance of cell shape and polarity, cell division and cytokinesis, vesicle and organelle movement, cell signalling as well as the establishment and maintenance of cell junctions, and regulation of transcription. It often takes place during the re-epithelialisation, where it is part of the proliferation phase. A report by Martin and Lewis (1992) mentioned that when embryonic wound occurred, the actin cable plays the role to close up the embryonic wound. The rapidly assembled actin purse string is responsible for the closure of the wound in the embryonic epidermis (Richardson et al., 2013). Therefore, actin plays an important role in embryogenesis as well as in wound healing, where cell motility is crucial for the sealing of wound margins. Other structural proteins also play a role in the remodelling of the tissue structure at the wound area. Actin works together with myosin in the wound healing process. The biomechanical process driving cell motility and relation between actin and myosin has been clearly demonstrated by Giannone et al. (2007). Conrad et al. (1993) published a report supporting that actin, myosin I and myosin II were involved in the wound healing process of fibroblasts. Myosin II can be regulated in order to accelerate the healing of large wounds and thus improving a wound in healing (Matsubayashi et al., 2011). Tropomyosin is a multi-isoform family of actin-associating proteins that controls isoform-specific regulation of diverse actin filaments (Bach et al., 2009; Gunning et al., 2005). A recent report by Lees et al. (2013) suggested that

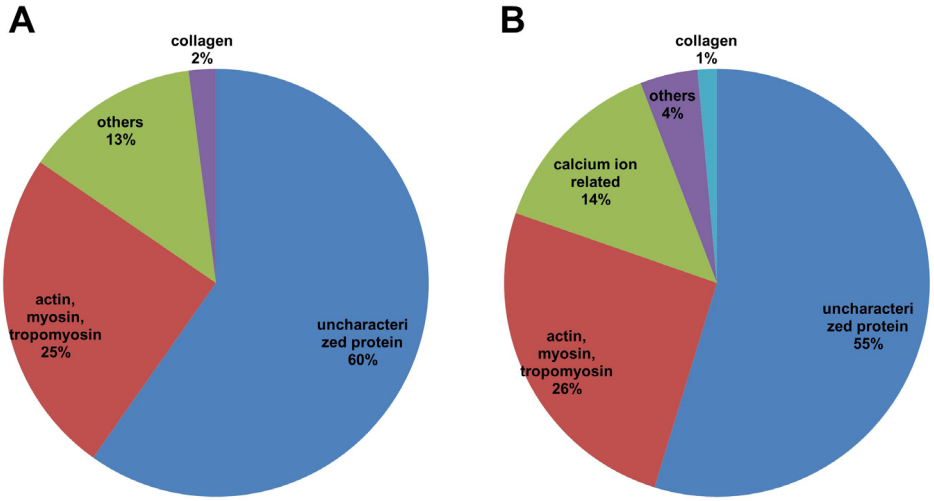

Figure 3: Distribution of protein that were identified by LCMS/MS from *C. striatus* samples

A: freeze-dried sample; **B**: spray-dried sample; The proteins were categorized into 5 groups: uncharacterised proteins; actin, myosin, tropomyosin; calcium ion-related proteins; collagen; others.

the tropomyosin may be important regulators of actin functioning during the wound healing process. In short, actin, myosin and tropomysosin are directly involved in the regulation and maintenance of wound recovery. Therefore, the high amount of myosin, actin and tropomyosin may give us the insight of the ability of accelerated wound healing properties promoted by the *C. striatus* application.

Calcium Ion-Related Protein

Another interesting finding obtained was related to the calcium ion-related protein detected in the samples. Ca^{2+} is known to play a role in directing cell polarity and guiding highly polarized processes like tip growth (Messerli and Robinson, 2007), neurite extension (Zheng and Poo, 2007), and cell migration (Maroto and Hamill, 2007). A research finding published by Graham et al. (2013) revealed that calcium signalling plays a role in inducing the cellular organization and also the migration of skin cells. The introduction of calcium ion would trigger the local fusion of internal membranes, creating a patch vesicle that staunches the wound (McNeil and Kirchhausen, 2005). However, Kono et al. (2012) has highlighted that the calcium ion entry has to be well-regulated because it is lethal in large amounts, but is required at moderate levels to induce the wound healing process. Such observation emphasized that calcium ion-related protein plays a significant role in regulating and initiating the process of wound healing once the wound is inflicted. However, the calcium transporting enzyme is only detected in spray-dried sample (Table 3) but not in the freeze-dried sample. The reason of such phenomenon is not clear and requires more extensive study.

Table 2: List of detected proteins in freeze-dried C. striatus samples from a combination of whole sample and fractions analysis

Accession	Max -10lgP	(%) Max coverage	Max # peptides	Max # unique	Description
tr|H2LNX8|H2LNX8_ORYLA	321.96	44	50	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=MYH13 (4 of 11) PE=4 SV=1
tr|Q05K09|Q05K09_ORYLA	320.56	23	50	0	Fast skeletal myosin heavy chain isoform mMYH-2 OS=Oryzias latipes PE=4 SV=1
tr|Q05K05|Q05K05_ORYLA	318.51	23	52	2	Fast skeletal myosin heavy chain isoform mMYH-11 OS=Oryzias latipes PE=4 SV=1
tr|Q05K11|Q05K11_ORYLA	312.54	22	45	0	Fast skeletal myosin heavy chain isoform mMYH-5 OS=Oryzias latipes PE=4 SV=1
tr|Q05K06|Q05K06_ORYLA	312.12	22	45	0	Fast skeletal myosin heavy chain mMYH-9 OS=Oryzias latipes PE=4 SV=1
tr|Q05K07|Q05K07_ORYLA	310.55	23	46	0	Fast skeletal myosin heavy chain isoform mMYH-7 OS=Oryzias latipes PE=4 SV=1
tr|G0YU50|G0YU50_SINCH	308.34	18	40	2	Fast skeletal muscle myosin heavy chain isoform 3 OS=Siniperca chuatsi PE=2 SV=1
tr|G0YU48|G0YU48_SINCH	308.34	18	40	2	Fast skeletal muscle myosin heavy chain isoform 1 OS=Siniperca chuatsi PE=2 SV=1
tr|G0YU49|G0YU49_SINCH	308.34	18	40	2	Fast skeletal muscle myosin heavy chain isoform 2 OS=Siniperca chuatsi PE=2 SV=1
tr|Q6SNT2|Q6SNT2_SINCH	308.34	18	40	2	Fast skeletal muscle myosin heavy chain OS=Siniperca chuatsi PE=2 SV=1
tr|H2LPK6|H2LPK6_ORYLA	307.58	20	45	0	Uncharacterized protein OS=Oryzias latipes GN=MYH13 (7 of 11) PE=4 SV=1
tr|Q05K10|Q05K10_ORYLA	307.16	20	45	0	Fast skeletal myosin heavy chain isoform mMYH-1 OS=Oryzias latipes PE=4 SV=1
tr|H2N1T3|H2N1T3_ORYLA	305.63	22	40	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=MYH13 (11 of 11) PE=4 SV=1
tr|H2LPD7|H2LPD7_ORYLA	305.52	20	39	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=MYH13 (6 of 11) PE=4 SV=1
tr|C4TIP2|C4TIP2_ORYLA	304.90	17	36	0	Myosin heavy chain larval type 1 OS=Oryzias latipes GN=mMYHL1 PE=2 SV=1
tr|Q05K12|Q05K12_ORYLA	304.48	21	42	0	Fast skeletal myosin heavy chain isoform mMYH-6 OS=Oryzias latipes PE=4 SV=1

tr|C4TIP1|C4TIP1_ORYLA	302.57	15	37	0	Myosin heavy chain embryonic type 1 OS=Oryzias latipes GN=mMYHemb1 PE=2 SV=1
tr|Q05K08|Q05K08_ORYLA	302.01	19	40	0	Fast skeletal myosin heavy chain isoform mMYH-3 OS=Oryzias latipes PE=4 SV=1
tr|C4TIP3|C4TIP3_ORYLA	301.82	16	35	0	Myosin heavy chain larval type 2 OS=Oryzias latipes GN=mMYHL2 PE=2 SV=1
tr|H2L9D3|H2L9D3_ORYLA	300.68	15	35	0	Uncharacterized protein OS=Oryzias latipes GN=LOC101160310 PE=4 SV=1
tr|H2L9E3|H2L9E3_ORYLA	300.68	15	35	0	Uncharacterized protein OS=Oryzias latipes GN=LOC101160310 PE=4 SV=1
tr|G1FCF2|G1FCF2_SINCH	284.42	81	46	2	Tropomyosin OS=Siniperca chuatsi PE=2 SV=1
tr|W5JYT8|W5JYT8_ASTMX	275.40	74	41	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2MA79|H2MA79_ORYLA	275.17	78	44	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101164789 PE=3 SV=1
tr|H2MCV5|H2MCV5_ORYLA	272.04	62	37	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101172748 PE=3 SV=1
tr|W5L2L4|W5L2L4_ASTMX	271.87	8	37	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5KS79|W5KS79_ASTMX	269.00	64	36	0	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5JZC9|W5JZC9_ASTMX	265.32	70	39	0	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5K3X6|W5K3X6_ASTMX	264.76	10	24	2	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2L9I1|H2L9I1_ORYLA	261.73	10	21	0	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|W5KTD3|W5KTD3_ASTMX	244.75	57	30	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|V9KFU2|V9KFU2_CALMI	242.28	52	26	0	Tropomyosin alpha-3 chain-like protein (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|V9KPC1|V9KPC1_CALMI	242.28	51	26	0	Tropomyosin1-1 (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|H2MRC9|H2MRC9_ORYLA	241.80	49	28	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101160801 PE=3 SV=1
tr|H2LUJ8|H2LUJ8_ORYLA	240.90	44	23	0	Uncharacterized protein (Fragment) OS=Oryzias latipes PE=3 SV=1
tr|W5L9M3|W5L9M3_ASTMX	240.03	10	19	3	Uncharacterized protein OS=Astyanax mexicanus GN=MYH13 (4 of 4) PE=4 SV=1
tr|V9K7A6|V9K7A6_CALMI	235.45	10	19	1	Myosin, heavy chain 7, cardiac muscle, beta OS=-Callorhynchus milii PE=2 SV=1
tr|W5K855|W5K855_ASTMX	222.23	9	18	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus GN=MYH13 (2 of 4) PE=4 SV=1
tr|D2KQG2|D2KQG2_SINCH	220.91	49	12	6	Parvalbumin 3 OS=Siniperca chuatsi PE=2 SV=1
tr|V9LBJ3|V9LBJ3_CALMI	219.92	38	18	0	Tropomyosin alpha-1 chain-like protein (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|H2MYK1|H2MYK1_ORYLA	215.55	59	15	0	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|H2MUU9|H2MUU9_ORYLA	214.46	30	19	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101167740 PE=3 SV=1

tr|H2MUU8|H2MUU8_ORYLA	214.46	29	19	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101167740 PE=3 SV=1
tr|V5RFJ8|V5RFJ8_SINCH	214.15	8	18	2	Slow skeletal muscle myosin heavy chain isoform 1 OS=Siniperca chuatsi PE=2 SV=1
tr|C6L8J0|C6L8J0_ORYLA	205.48	7	17	1	Ventricular myosin heavy chain OS=Oryzias latipes GN=vmhc PE=2 SV=1
tr|H2MDF6|H2MDF6_ORYLA	205.48	7	17	1	Uncharacterized protein OS=Oryzias latipes GN=vmhc PE=4 SV=1
tr|W5KA98|W5KA98_ASTMX	200.86	8	15	0	Uncharacterized protein OS=Astyanax mexicanus GN=MYH13 (3 of 4) PE=4 SV=1
tr|W5L7Y3|W5L7Y3_ASTMX	198.60	33	21	0	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5KAS6|W5KAS6_ASTMX	195.33	38	20	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2L9Z5|H2L9Z5_ORYLA	186.03	48	8	4	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=mylz2 PE=4 SV=1
tr|W5KKQ1|W5KKQ1_ASTMX	184.10	54	10	3	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2LNN2|H2LNN2_ORYLA	175.16	5	13	0	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|V9KCZ9|V9KCZ9_CALMI	174.06	7	10	0	Slow myosin heavy chain 2 (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|G8GWA4|G8GWA4_SINCH	168.04	46	7	0	Parvalbumin 1 OS=Siniperca chuatsi PE=2 SV=2
tr|A8QX86|A8QX86_ORYLA	143.29	5	6	4	Collagen type I alpha 1 OS=Oryzias latipes GN=COL1 PE=2 SV=1
tr|H2MRA6|H2MRA6_ORYLA	143.29	5	6	4	Uncharacterized protein OS=Oryzias latipes GN=col1 PE=4 SV=1
tr|B7U3X4|B7U3X4_SINKN	137.22	32	5	1	Myosin light chain 2 OS=Siniperca knerii GN=MCL PE=2 SV=1
tr|B9VJM4|B9VJM4_SINCH	134.35	28	4	4	Troponin C OS=Siniperca chuatsi PE=2 SV=1
tr|H2L8R0|H2L8R0_ORYLA	134.35	28	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101171829 PE=4 SV=1
tr|H2L8Q7|H2L8Q7_ORYLA	134.35	28	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101171829 PE=4 SV=1
tr|H2L8Q9|H2L8Q9_ORYLA	134.35	28	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101171829 PE=4 SV=1
tr|B9VJM3|B9VJM3_SINCH	129.26	44	6	3	Parvalbumin OS=Siniperca chuatsi PE=2 SV=1
tr|W5LN49|W5LN49_ASTMX	116.63	35	4	4	Uncharacterized protein OS=Astyanax mexicanus GN=ACTL8 PE=3 SV=1
tr|Q9PSV5|Q9PSV5_ORYLA	116.63	29	4	4	Skeletal muscle actin (Fragment) OS=Oryzias latipes GN=OlMA1 PE=3 SV=1
tr|W5KS21|W5KS21_ASTMX	116.63	14	4	4	Uncharacterized protein OS=Astyanax mexicanus GN=ACTG1 (2 of 2) PE=3 SV=1
tr|W5KBE9|W5KBE9_ASTMX	116.63	12	4	4	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4GLR4|K4GLR4_CALMI	116.63	12	4	4	Cytoskeletal beta actin OS=Callorhynchus milii PE=2 SV=1
tr|H2MSI7|H2MSI7_ORYLA	116.63	12	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101156021 PE=3 SV=1
tr|K4G324|K4G324_CALMI	116.63	12	4	4	Actin, cytoplasmic 1 OS=Callorhynchus milii PE=2 SV=1

tr|K4FTZ1|K4FTZ1_CALMI	116.63	12	4	4	Actin, cytoplasmic 1 OS=Callorhynchus milii PE=2 SV=1
sp|P79818|ACTB_ORYLA	116.63	12	4	4	Actin, cytoplasmic 1 OS=Oryzias latipes GN=actb PE=2 SV=2
tr|K4G4I8|K4G4I8_CALMI	116.63	12	4	4	Actin, cytoplasmic 1 OS=Callorhynchus milii PE=2 SV=1
tr|G7Z090|G7Z090_SINCH	116.63	12	4	4	Beta-actin OS=Siniperca chuatsi GN=ACTb PE=3 SV=1
tr|H2LZV3|H2LZV3_ORYLA	116.63	12	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101168921 PE=3 SV=1
tr|W5K9V8|W5K9V8_ASTMX	116.63	12	4	4	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4GFR7|K4GFR7_CALMI	116.63	12	4	4	Actin, cytoplasmic 1 OS=Callorhynchus milii PE=2 SV=1
tr|K4GIQ3|K4GIQ3_CALMI	116.63	12	4	4	Beta-actin OS=Callorhynchus milii PE=2 SV=1
tr|W5KBE8|W5KBE8_ASTMX	116.63	12	4	4	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5KQ47|W5KQ47_ASTMX	116.63	12	4	4	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4G587|K4G587_CALMI	116.63	12	4	4	Actin, alpha 2, smooth muscle, aorta OS=Callorhynchus milii PE=2 SV=1
tr|Q6TKP3|Q6TKP3_SINCH	116.63	12	4	4	Skeletal muscle alpha-actin OS=Siniperca chuatsi PE=2 SV=1
tr|K4GDF4|K4GDF4_CALMI	116.63	12	4	4	Actin, alpha 2 OS=Callorhynchus milii PE=2 SV=1
tr|Q76N20|Q76N20_ORYLA	116.63	12	4	4	Cardiac muscle actin OS=Oryzias latipes GN=OlMA1 PE=3 SV=1
tr|K4GEN0|K4GEN0_CALMI	116.63	12	4	4	Actin, alpha 2 OS=Callorhynchus milii PE=2 SV=1
tr|W5K0Q7|W5K0Q7_ASTMX	116.63	12	4	4	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5K7M9|W5K7M9_ASTMX	116.63	12	4	4	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2MU18|H2MU18_ORYLA	116.63	12	4	4	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101170382 PE=3 SV=1
tr|K4FYQ2|K4FYQ2_CALMI	116.63	11	4	4	Beta-actin OS=Callorhynchus milii PE=2 SV=1
tr|W5K1N1|W5K1N1_ASTMX	115.42	11	5	3	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5LD34|W5LD34_ASTMX	115.42	10	5	3	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=3 SV=1
tr|H2LHF2|H2LHF2_ORYLA	109.74	6	9	8	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|W5L8R7|W5L8R7_ASTMX	108.61	4	6	4	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5KKR2|W5KKR2_ASTMX	108.23	46	5	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2M0U0|H2M0U0_ORYLA	104.86	44	5	0	Uncharacterized protein OS=Oryzias latipes GN=LOC101165806 PE=4 SV=1
tr|W5LNC0|W5LNC0_ASTMX	95.18	29	4	0	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
tr|W5LPS5|W5LPS5_ASTMX	93.27	18	3	2	Uncharacterized protein OS=Astyanax mexicanus GN=MYL3 PE=4 SV=1

tr|W5LNC1|W5LNC1_ASTMX	89.82	23	4	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2MMQ8|H2MMQ8_ORYLA	86.08	15	3	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101167500 PE=4 SV=1
tr|B7U3X3|B7U3X3_SINKN	86.08	15	3	1	Myosin light chain 1 OS=Siniperca knerii GN=MCL PE=2 SV=1
tr|C7EP35|C7EP35_SINSC	86.08	15	3	1	Myosin light chain 1 OS=Siniperca scherzeri PE=2 SV=1
tr|B6VCB3|B6VCB3_SINKN	84.68	24	4	2	Myosin light chain 3 OS=Siniperca knerii PE=2 SV=1
tr|H2MBE4|H2MBE4_ORYLA	82.41	5	2	2	Uncharacterized protein (Fragment) OS=Oryzias latipes PE=3 SV=1
tr|W5LI76|W5LI76_ASTMX	79.33	8	2	2	Glyceraldehyde-3-phosphate dehydrogenase OS=Astyanax mexicanus PE=3 SV=1
tr|W5LPK3|W5LPK3_ASTMX	78.29	2	3	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
tr|H2MXN4|H2MXN4_ORYLA	77.13	6	2	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101175132 PE=3 SV=1
tr|H2MXN1|H2MXN1_ORYLA	77.13	6	2	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101175132 PE=3 SV=1
tr|W5LLK3|W5LLK3_ASTMX	77.13	6	2	2	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2MXM8|H2MXM8_ORYLA	77.13	6	2	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101175132 PE=3 SV=1
tr|G8GWA3|G8GWA3_SINCH	76.44	10	2	1	Parvalbumin 2 OS=Siniperca chuatsi PE=2 SV=2
tr|H2M0U7|H2M0U7_ORYLA	75.86	23	3	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101166553 PE=4 SV=1
tr|W5JXT6|W5JXT6_ASTMX	75.12	1	2	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2M6N2|H2M6N2_ORYLA	73.70	2	3	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101162163 PE=4 SV=1
tr|W5KH68|W5KH68_ASTMX	73.70	20	3	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5K3P7|W5K3P7_ASTMX	73.05	1	3	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2MW80|H2MW80_ORYLA	71.85	19	2	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101161287 PE=3 SV=1
tr|W5K5Z0|W5K5Z0_ASTMX	68.52	1	2	2	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|V9K7D3|V9K7D3_CALMI	66.16	1	1	1	Collagen alpha-1(I) chain OS=Callorhynchus milii PE=2 SV=1
tr|W5K0S8|W5K0S8_ASTMX	60.65	1	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5KAN3|W5KAN3_ASTMX	59.59	6	3	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2MNV5|H2MNV5_ORYLA	59.26	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101166392 PE=3 SV=1
tr|W5KDD1|W5KDD1_ASTMX	59.26	4	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=3 SV=1
tr|Q6XE27|Q6XE27_CONER	59.26	66	1	1	Tubulin (Fragment) OS=Conus ermineus PE=4 SV=1

tr|Q65CK3|Q65CK3_CONTU	59.26	24	1	1	Beta tubulin (Fragment) OS=Conus tulipa PE=3 SV=1
tr|W5KBI3|W5KBI3_ASTMX	59.26	5	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus GN=TUBB2A PE=3 SV=1
tr|W5LD44|W5LD44_ASTMX	59.26	4	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus GN=TUBB (1 of 2) PE=3 SV=1
tr|W5LI88|W5LI88_ASTMX	59.26	4	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|V9KI87|V9KI87_CALMI	59.26	4	1	1	Tubulin beta chain-like protein OS=Callorhynchus milii PE=2 SV=1
tr|H2MCJ1|H2MCJ1_ORYLA	59.26	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101157969 PE=3 SV=1
tr|K4FSH1|K4FSH1_CALMI	59.26	4	1	1	Beta1-tubulin OS=Callorhynchus milii PE=2 SV=1
tr|Q8UUK8|Q8UUK8_ORYLA	59.26	4	1	1	TUBB protein OS=Oryzias latipes GN=TUBB PE=3 SV=1
tr|K4G4H2|K4G4H2_CALMI	59.26	4	1	1	Beta1-tubulin OS=Callorhynchus milii PE=2 SV=1
tr|W5LKB5|W5LKB5_ASTMX	59.26	4	1	1	Uncharacterized protein OS=Astyanax mexicanus GN=TUBB (2 of 2) PE=3 SV=1
tr|H2LS01|H2LS01_ORYLA	59.26	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101164232 PE=3 SV=1
tr|W5KZP7|W5KZP7_ASTMX	59.26	4	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4GIQ2|K4GIQ2_CALMI	59.26	4	1	1	Tubulin, beta 2C OS=Callorhynchus milii PE=2 SV=1
tr|H2LRN0|H2LRN0_ORYLA	59.26	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=tubb PE=3 SV=1
tr|H2MTT9|H2MTT9_ORYLA	59.26	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=TUBB4A PE=3 SV=1
tr|K4FTV1|K4FTV1_CALMI	59.26	4	1	1	Tubulin, beta 2C OS=Callorhynchus milii PE=2 SV=1
tr|H2LEF2|H2LEF2_ORYLA	59.26	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101159143 PE=3 SV=1
tr|W5KEC5|W5KEC5_ASTMX	59.26	4	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=3 SV=1
tr|W5K7N6|W5K7N6_ASTMX	59.26	4	1	1	Uncharacterized protein OS=Astyanax mexicanus GN=TUBB3 PE=3 SV=1
tr|W5L9L2|W5L9L2_ASTMX	59.26	4	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=3 SV=1
tr|H2MPL0|H2MPL0_ORYLA	55.44	2	1	1	Serotransferrin OS=Oryzias latipes GN=LOC100144362 PE=3 SV=1
tr|A8MN21|A8MN21_ORYLA	55.44	2	1	1	Serotransferrin OS=Oryzias latipes PE=2 SV=1
sp|P79819|TRFE_ORYLA	55.44	2	1	1	Serotransferrin OS=Oryzias latipes GN=tf PE=3 SV=1
tr|H2L7M4|H2L7M4_ORYLA	54.51	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101168952 PE=3 SV=1
tr|H2L7M3|H2L7M3_ORYLA	54.51	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101168952 PE=3 SV=1
tr|H2L7L7|H2L7L7_ORYLA	54.51	4	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101168952 PE=3 SV=1

tr|H2LPB8|H2LPB8_ORYLA	53.97	7	2	2	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101170788 PE=3 SV=1
tr|W5LSN2|W5LSN2_ASTMX	50.91	4	1	0	Uncharacterized protein OS=Astyanax mexicanus GN=MYL4 (2 of 3) PE=4 SV=1
tr|H2MKG7|H2MKG7_ORYLA	48.00	7	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101170317 PE=4 SV=1
tr|H2L4J5|H2L4J5_ORYLA	45.96	6	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101155923 PE=4 SV=1
tr|H2L4J9|H2L4J9_ORYLA	45.96	5	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101155923 PE=4 SV=1
tr|B9VJM2|B9VJM2_SINCH	45.96	5	1	1	Troponin I OS=Siniperca chuatsi PE=2 SV=1
tr|H2L4K6|H2L4K6_ORYLA	45.96	5	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101156490 PE=4 SV=1
tr|H2L4I4|H2L4I4_ORYLA	45.96	5	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101155682 PE=4 SV=1
tr|H2L4I0|H2L4I0_ORYLA	45.96	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101155425 PE=4 SV=1
tr|Q8JIP9|Q8JIP9_ORYLA	44.31	3	1	1	Warm-temperature-acclimation-related-65 OS=Oryzias latipes GN=wap65 PE=2 SV=1
tr|H2M355|H2M355_ORYLA	44.31	3	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=wap65 PE=4 SV=1
tr|W5LLP2|W5LLP2_ASTMX	42.44	6	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2LBD5|H2LBD5_ORYLA	42.44	6	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101164670 PE=4 SV=1
tr|W5LRW3|W5LRW3_ASTMX	42.44	11	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|F5ANJ2|F5ANJ2_SINCH	42.44	10	1	1	Ubiquitin a (Fragment) OS=Siniperca chuatsi PE=2 SV=1
tr|V9LHV4|V9LHV4_CALMI	42.44	9	1	1	Polyubiquitin-C-like protein (Fragment) OS=-Callorhynchus milii PE=2 SV=1
tr|W5LI91|W5LI91_ASTMX	42.44	9	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
tr|Q2KKX3|Q2KKX3_SINCH	42.44	8	1	1	Ubiquitin (Fragment) OS=Siniperca chuatsi PE=2 SV=1
tr|H2LX76|H2LX76_ORYLA	42.44	7	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101169681 PE=4 SV=1
tr|E2I6H0|E2I6H0_SINCH	42.44	7	1	1	Ubiquitin OS=Siniperca chuatsi PE=2 SV=1
tr|K4G4C1|K4G4C1_CALMI	42.44	7	1	1	Ribosomal protein L40-like isoform 1 OS=Callorhynchus milii PE=2 SV=1
tr|H2L6M8|H2L6M8_ORYLA	42.44	4	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101167305 PE=4 SV=1
tr|W5LLW5|W5LLW5_ASTMX	42.44	2	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|K4FTX6|K4FTX6_CALMI	42.44	2	1	1	Polyubiquitin-like protein OS=Callorhynchus milii PE=2 SV=1
tr|H2M983|H2M983_ORYLA	42.44	1	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101172100 PE=4 SV=1
tr|W5KHS4|W5KHS4_ASTMX	38.98	10	1	1	Uncharacterized protein OS=Astyanax mexicanus GN=MYL2 (2 of 3) PE=4 SV=1
tr|H2MMN3|H2MMN3_ORYLA	38.98	8	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101162219 PE=4 SV=1

tr|H2MMN4|H2MMN4_ORYLA	38.98	8	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101162219 PE=4 SV=1
tr|H2LZ88|H2LZ88_ORYLA	38.98	7	1	1	Uncharacterized protein OS=Oryzias latipes GN=MYL2 (1 of 2) PE=4 SV=1
tr|W5L038|W5L038_ASTMX	38.42	0	1	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|K4FV77|K4FV77_CALMI	35.11	6	1	1	MLC1f/3f OS=Callorhynchus milii PE=2 SV=1
tr|V9KS09|V9KS09_CALMI	35.11	6	1	1	Myosin light chain 1/3, skeletal muscle isoform isoform 3f OS=Callorhynchus milii PE=2 SV=1
tr|V9LGV1|V9LGV1_CALMI	35.11	6	1	1	Myosin light polypeptide 6B OS=Callorhynchus milii PE=2 SV=1
tr|V9LFC7|V9LFC7_CALMI	35.11	6	1	1	Myosin, light chain 6, alkali, smooth muscle and non-muscle OS=Callorhynchus milii PE=2 SV=1
tr|V9L5Z0|V9L5Z0_CALMI	35.11	5	1	1	Myosin light chain 1/3, skeletal muscle isoform isoform 3f (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|V9L470|V9L470_CALMI	35.11	5	1	1	Myosin, light chain 4, alkali OS=Callorhynchus milii PE=2 SV=1
tr|V9KMS0|V9KMS0_CALMI	35.11	5	1	1	Myosin, light chain 1, alkali OS=Callorhynchus milii PE=2 SV=1
tr|H2MT93|H2MT93_ORYLA	35.11	5	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101157706 PE=4 SV=1
tr|H2MTE5|H2MTE5_ORYLA	35.11	5	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101157250 PE=4 SV=1
tr|W5KSS8|W5KSS8_ASTMX	35.11	4	1	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2M7W2|H2M7W2_ORYLA	30.87	9	1	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101174831 PE=4 SV=1
tr|H2LK91|H2LK91_ORYLA	30.63	0	0	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=col2a1a PE=4 SV=1
tr|G1UH59|G1UH59_ORYLA	30.63	0	0	0	Type II collagen A isoform 2 OS=Oryzias latipes GN=col2a1a PE=2 SV=1
tr|H2LK90|H2LK90_ORYLA	30.63	0	0	0	Uncharacterized protein OS=Oryzias latipes GN=col2a1a PE=4 SV=1
tr|G1UH58|G1UH58_ORYLA	30.63	0	0	0	Type II collagen A isoform 1 OS=Oryzias latipes GN=col2a1a PE=2 SV=1
tr|W5LKA3|W5LKA3_ASTMX	30.03	1	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
total 194 proteins					

Table 3: List of detected proteins in spray-dried *C. striatus* samples from a combination of whole sample and fractions analysis

Accession	Max -10lgP	(%) Max coverage	Max # peptides	Max # unique	Description
tr|Q05K05|Q05K05_ORYLA	187.31	33	108	2	Fast skeletal myosin heavy chain isoform mMYH-11 OS=Oryzias latipes PE=4 SV=1
tr|H2LNX8|H2LNX8_ORYLA	185.20	61	106	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=MYH13 (4 of 11) PE=4 SV=1
tr|Q05K07|Q05K07_ORYLA	184.06	31	101	0	Fast skeletal myosin heavy chain isoform mMYH-7 OS=Oryzias latipes PE=4 SV=1
tr|Q6SNT2|Q6SNT2_SINCH	184.03	29	96	6	Fast skeletal muscle myosin heavy chain OS=Siniperca chuatsi PE=2 SV=1

tr|G0YU48|G0YU48_SINCH	184.03	29	96	6	Fast skeletal muscle myosin heavy chain isoform 1 OS=Siniperca chuatsi PE=2 SV=1
tr|G0YU49|G0YU49_SINCH	184.03	29	96	6	Fast skeletal muscle myosin heavy chain isoform 2 OS=Siniperca chuatsi PE=2 SV=1
tr|G0YU50|G0YU50_SINCH	184.03	29	96	6	Fast skeletal muscle myosin heavy chain isoform 3 OS=Siniperca chuatsi PE=2 SV=1
tr|Q05K09|Q05K09_ORYLA	183.87	33	106	0	Fast skeletal myosin heavy chain isoform mMYH-2 OS=Oryzias latipes PE=4 SV=1
tr|Q05K08|Q05K08_ORYLA	182.40	29	95	0	Fast skeletal myosin heavy chain isoform mMYH-3 OS=Oryzias latipes PE=4 SV=1
tr|H2LPD7|H2LPD7_ORYLA	182.07	29	93	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=MYH13 (6 of 11) PE=4 SV=1
tr|Q05K06|Q05K06_ORYLA	181.98	32	101	0	Fast skeletal myosin heavy chain mMYH-9 OS=Oryzias latipes PE=4 SV=1
tr|Q05K11|Q05K11_ORYLA	181.58	32	100	0	Fast skeletal myosin heavy chain isoform mMYH-5 OS=Oryzias latipes PE=4 SV=1
tr|H2N1T3|H2N1T3_ORYLA	181.41	32	92	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=MYH13 (11 of 11) PE=4 SV=1
tr|H2LPK6|H2LPK6_ORYLA	179.79	30	97	0	Uncharacterized protein OS=Oryzias latipes GN=MYH13 (7 of 11) PE=4 SV=1
tr|Q05K12|Q05K12_ORYLA	179.40	30	94	0	Fast skeletal myosin heavy chain isoform mMYH-6 OS=Oryzias latipes PE=4 SV=1
tr|Q05K10|Q05K10_ORYLA	178.52	29	92	0	Fast skeletal myosin heavy chain isoform mMYH-1 OS=Oryzias latipes PE=4 SV=1
tr|C4TIP2|C4TIP2_ORYLA	178.34	26	81	0	Myosin heavy chain larval type 1 OS=Oryzias latipes GN=mMYHL1 PE=2 SV=1
tr|C4TIP3|C4TIP3_ORYLA	178.24	27	81	0	Myosin heavy chain larval type 2 OS=Oryzias latipes GN=mMYHL2 PE=2 SV=1
tr|C4TIP1|C4TIP1_ORYLA	177.92	26	90	0	Myosin heavy chain embryonic type 1 OS=Oryzias latipes GN=mMYHemb1 PE=2 SV=1
tr|H2L9D3|H2L9D3_ORYLA	176.38	24	84	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101160310 PE=4 SV=1
tr|H2L9E3|H2L9E3_ORYLA	176.38	24	84	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101160310 PE=4 SV=1
tr|W5L2L4|W5L2L4_ASTMX	173.01	13	82	2	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|V9K7A6|V9K7A6_CALMI	161.02	18	66	6	Myosin, heavy chain 7, cardiac muscle, beta OS=Callorhynchus milii PE=2 SV=1
tr|W5K3X6|W5K3X6_ASTMX	158.95	19	57	5	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2L9I1|H2L9I1_ORYLA	157.53	19	52	0	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|W5L9M3|W5L9M3_ASTMX	148.03	15	44	4	Uncharacterized protein OS=Astyanax mexicanus GN=MYH13 (4 of 4) PE=4 SV=1
tr|W5L0R5|W5L0R5_ASTMX	146.58	10	39	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|V5RFJ8|V5RFJ8_SINCH	146.10	13	44	3	Slow skeletal muscle myosin heavy chain isoform 1 OS=Siniperca chuatsi PE=2 SV=1
tr|G1FCF2|G1FCF2_SINCH	144.79	75	48	3	Tropomyosin OS=Siniperca chuatsi PE=2 SV=1

tr|C6L8J0|C6L8J0_ORYLA	141.71	11	37	0	Ventricular myosin heavy chain OS=Oryzias latipes GN=vmhc PE=2 SV=1
tr|H2MDF6|H2MDF6_ORYLA	141.71	11	37	0	Uncharacterized protein OS=Oryzias latipes GN=vmhc PE=4 SV=1
tr|H2MA79|H2MA79_ORYLA	141.21	69	45	0	Uncharacterized protein OS=Oryzias latipes GN=LOC101164789 PE=3 SV=1
tr|W5JYT8|W5JYT8_ASTMX	139.36	71	41	1	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5KA98|W5KA98_ASTMX	136.71	11	30	0	Uncharacterized protein OS=Astyanax mexicanus GN=MYH13 (3 of 4) PE=4 SV=1
tr|H2MDC8|H2MDC8_ORYLA	135.02	12	37	1	Uncharacterized protein OS=Oryzias latipes GN=mmyhc1 PE=4 SV=1
tr|W5K2C5|W5K2C5_ASTMX	134.18	9	27	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2MCV5|H2MCV5_ORYLA	132.50	68	36	8	Uncharacterized protein OS=Oryzias latipes GN=LOC101172748 PE=3 SV=1
tr|W5KS79|W5KS79_ASTMX	131.30	55	32	0	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2MYK1|H2MYK1_ORYLA	130.16	79	25	0	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|W5K855|W5K855_ASTMX	129.27	12	29	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus GN=MYH13 (2 of 4) PE=4 SV=1
tr|V9KFU2|V9KFU2_CALMI	128.41	58	30	1	Tropomyosin alpha-3 chain-like protein (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|V9KPC1|V9KPC1_CALMI	128.41	57	30	1	Tropomyosin1-1 (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|H2LNN2|H2LNN2_ORYLA	126.92	12	37	0	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|H2MLX5|H2MLX5_ORYLA	126.82	9	30	0	Uncharacterized protein OS=Oryzias latipes GN=LOC101160127 PE=4 SV=1
tr|V9KCZ9|V9KCZ9_CALMI	126.60	19	34	0	Slow myosin heavy chain 2 (Fragment) OS=-Callorhynchus milii PE=2 SV=1
tr|H2MRC9|H2MRC9_ORYLA	125.16	53	30	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101160801 PE=3 SV=1
tr|V9LBJ3|V9LBJ3_CALMI	111.64	40	18	0	Tropomyosin alpha-1 chain-like protein (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|H2L9Z5|H2L9Z5_ORYLA	109.49	70	14	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=mylz2 PE=4 SV=1
tr|V9KBQ3|V9KBQ3_CALMI	109.08	10	21	0	Myosin-7B-like protein (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|W5L7Y3|W5L7Y3_ASTMX	105.31	31	20	0	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5KKQ1|W5KKQ1_ASTMX	102.15	53	13	5	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5KBE9|W5KBE9_ASTMX	102.08	25	14	14	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4GDF4|K4GDF4_CALMI	102.08	23	14	14	Actin, alpha 2 OS=Callorhynchus milii PE=2 SV=1
tr|W5KBE8|W5KBE8_ASTMX	102.08	23	14	14	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1

tr|Q76N20|Q76N20_ORYLA	102.08	23	14	14	Cardiac muscle actin OS=Oryzias latipes GN=OlMA1 PE=3 SV=1
tr|W5KQ47|W5KQ47_ASTMX	102.08	23	14	14	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4GEN0|K4GEN0_CALMI	102.08	23	14	14	Actin, alpha 2 OS=Callorhynchus milii PE=2 SV=1
tr|W5K0Q7|W5K0Q7_ASTMX	102.08	23	14	14	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
sp|Q98972|ACTS_ORYLA	102.08	23	14	14	Actin, alpha skeletal muscle OS=Oryzias latipes GN=acta1 PE=2 SV=1
tr|Q6TKP3|Q6TKP3_SINCH	102.08	23	14	14	Skeletal muscle alpha-actin OS=Siniperca chuatsi PE=2 SV=1
tr|W5K7M9|W5K7M9_ASTMX	102.08	23	14	14	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|K4G587|K4G587_CALMI	102.08	23	14	14	Actin, alpha 2, smooth muscle, aorta OS=Callorhynchus milii PE=2 SV=1
tr|H2MU18|H2MU18_ORYLA	102.08	23	14	14	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101170382 PE=3 SV=1
tr|D2KQG2|D2KQG2_SINCH	99.67	49	10	4	Parvalbumin 3 OS=Siniperca chuatsi PE=2 SV=1
tr|W5L0H6|W5L0H6_ASTMX	97.77	35	15	2	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5KT30|W5KT30_ASTMX	96.00	52	11	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|B7U3X4|B7U3X4_SINKN	94.15	66	11	2	Myosin light chain 2 OS=Siniperca knerii GN=MCL PE=2 SV=1
tr|W5K709|W5K709_ASTMX	92.83	44	10	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|G8GWA4|G8GWA4_SINCH	87.40	58	8	1	Parvalbumin 1 OS=Siniperca chuatsi PE=2 SV=2
tr|H2MRA6|H2MRA6_ORYLA	76.88	7	10	7	Uncharacterized protein OS=Oryzias latipes GN=col1 PE=4 SV=1
tr|A8QX86|A8QX86_ORYLA	76.88	7	10	7	Collagen type I alpha 1 OS=Oryzias latipes GN=COL1 PE=2 SV=1
tr|H2LHF2|H2LHF2_ORYLA	69.38	7	12	11	Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
tr|B9VJM4|B9VJM4_SINCH	66.94	24	4	4	Troponin C OS=Siniperca chuatsi PE=2 SV=1
tr|H2L8R0|H2L8R0_ORYLA	66.94	24	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101171829 PE=4 SV=1
tr|H2L8Q7|H2L8Q7_ORYLA	66.94	24	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101171829 PE=4 SV=1
tr|H2L8Q9|H2L8Q9_ORYLA	66.94	24	4	4	Uncharacterized protein OS=Oryzias latipes GN=LOC101171829 PE=4 SV=1
tr|W5L8R7|W5L8R7_ASTMX	64.09	4	9	4	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5LNC1|W5LNC1_ASTMX	61.90	24	5	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2MXN4|H2MXN4_ORYLA	61.74	6	3	3	Uncharacterized protein OS=Oryzias latipes GN=LOC101175132 PE=3 SV=1
tr|H2MXN1|H2MXN1_ORYLA	61.74	6	3	3	Uncharacterized protein OS=Oryzias latipes GN=LOC101175132 PE=3 SV=1

tr|W5LLK3|W5LLK3_ASTMX	61.74	6	3	3	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|H2MXM8|H2MXM8_ORYLA	61.74	6	3	3	Uncharacterized protein OS=Oryzias latipes GN=LOC101175132 PE=3 SV=1
tr|W5KKR2|W5KKR2_ASTMX	60.48	47	6	0	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|W5KHS4|W5KHS4_ASTMX	59.79	26	3	0	Uncharacterized protein OS=Astyanax mexicanus GN=MYL2 (2 of 3) PE=4 SV=1
tr|W5LI76|W5LI76_ASTMX	59.21	8	3	3	Glyceraldehyde-3-phosphate dehydrogenase OS=Astyanax mexicanus PE=3 SV=1
tr|H2M0U0|H2M0U0_ORYLA	58.82	44	5	0	Uncharacterized protein OS=Oryzias latipes GN=LOC101165806 PE=4 SV=1
tr|B6VCB3|B6VCB3_SINKN	57.86	27	5	2	Myosin light chain 3 OS=Siniperca knerii PE=2 SV=1
tr|W5LPS5|W5LPS5_ASTMX	54.69	18	3	3	Uncharacterized protein OS=Astyanax mexicanus GN=MYL3 PE=4 SV=1
tr|W5JXT6|W5JXT6_ASTMX	54.46	3	6	3	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|B9VJM3|B9VJM3_SINCH	54.34	41	5	1	Parvalbumin OS=Siniperca chuatsi PE=2 SV=1
tr|W5LNC0|W5LNC0_ASTMX	53.48	29	4	0	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
tr|W5LPK3|W5LPK3_ASTMX	53.14	2	4	2	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
tr|H2MMQ8|H2MMQ8_ORYLA	52.72	19	4	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101167500 PE=4 SV=1
tr|B7U3X3|B7U3X3_SINKN	52.72	19	4	1	Myosin light chain 1 OS=Siniperca knerii GN=MCL PE=2 SV=1
tr|C7EP35|C7EP35_SINSC	52.72	19	4	1	Myosin light chain 1 OS=Siniperca scherzeri PE=2 SV=1
tr|V9KUX9|V9KUX9_CALMI	51.39	15	3	3	Triosephosphate isomerase OS=Callorhynchus milii PE=2 SV=1
tr|H2MB61|H2MB61_ORYLA	51.39	14	3	3	Triosephosphate isomerase (Fragment) OS=Oryzias latipes GN=tpi-b PE=3 SV=1
tr|V9L1I9|V9L1I9_CALMI	49.53	12	3	3	Troponin T type 3 (Skeletal, fast) OS=Callorhynchus milii PE=2 SV=1
tr|W5K1N1|W5K1N1_ASTMX	47.89	4	2	2	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5LD34|W5LD34_ASTMX	47.89	4	2	2	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=3 SV=1
tr|H2MBE4|H2MBE4_ORYLA	46.20	5	2	2	Uncharacterized protein (Fragment) OS=Oryzias latipes PE=3 SV=1
tr|V9LAW6|V9LAW6_CALMI	45.28	18	2	1	Myosin light chain 2V (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|H2M0U7|H2M0U7_ORYLA	44.36	24	4	0	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101166553 PE=4 SV=1
tr|W5KH68|W5KH68_ASTMX	42.82	20	4	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2LAV5|H2LAV5_ORYLA	41.98	21	4	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101165624 PE=4 SV=1

tr|W5K3M6|W5K3M6_ASTMX	41.88	4	3	3	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5K3P7|W5K3P7_ASTMX	41.65	1	3	1	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2M6N2|H2M6N2_ORYLA	39.68	2	5	1	Uncharacterized protein OS=Oryzias latipes GN=LOC101162163 PE=4 SV=1
tr|G8GWA3|G8GWA3_SINCH	38.87	10	2	1	Parvalbumin 2 OS=Siniperca chuatsi PE=2 SV=2
tr|V9K8V8|V9K8V8_CALMI	37.44	2	1	1	Calcium-transporting ATPase OS=Callorhynchus milii PE=2 SV=1
tr|H2LN45|H2LN45_ORYLA	37.44	2	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=LOC101171864 PE=3 SV=1
tr|H2LN49|H2LN49_ORYLA	37.44	2	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=LOC101171864 PE=3 SV=1
tr|W5LFK4|W5LFK4_ASTMX	37.44	5	1	1	Uncharacterized protein OS=Astyanax mexicanus GN=ATP2A1 (1 of 2) PE=4 SV=1
tr|H2M9N1|H2M9N1_ORYLA	37.44	3	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=ATP2A1 (2 of 2) PE=3 SV=1
tr|V9KH80|V9KH80_CALMI	37.44	2	1	1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2-like protein (Fragment) OS=Callorhynchus milii PE=2 SV=1
tr|W5LM60|W5LM60_ASTMX	37.44	2	1	1	Uncharacterized protein OS=Astyanax mexicanus GN=ATP2A1 (2 of 2) PE=4 SV=1
tr|W5KSD8|W5KSD8_ASTMX	37.44	2	1	1	Uncharacterized protein (Fragment) OS=Astyanax mexicanus PE=4 SV=1
tr|G1EJ40|G1EJ40_SINCH	37.44	2	1	1	Calcium-transporting ATPase OS=Siniperca chuatsi PE=2 SV=1
tr|H2M858|H2M858_ORYLA	37.44	2	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101171024 PE=4 SV=1
tr|H2M854|H2M854_ORYLA	37.44	2	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101171024 PE=4 SV=1
tr|W5L8A3|W5L8A3_ASTMX	37.44	2	1	1	Calcium-transporting ATPase OS=Astyanax mexicanus PE=3 SV=1
tr|V9KCE0|V9KCE0_CALMI	37.44	2	1	1	Calcium-transporting ATPase OS=Callorhynchus milii PE=2 SV=1
tr|H2M9M8|H2M9M8_ORYLA	37.44	2	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=ATP2A1 (2 of 2) PE=3 SV=1
tr|H2MMF8|H2MMF8_ORYLA	37.44	2	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=LOC101160819 PE=3 SV=1
tr|H2M9N3|H2M9N3_ORYLA	37.44	2	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=ATP2A1 (2 of 2) PE=3 SV=1
tr|H2MMF9|H2MMF9_ORYLA	37.44	2	1	1	Calcium-transporting ATPase OS=Oryzias latipes GN=LOC101160819 PE=3 SV=1
tr|W5LA91|W5LA91_ASTMX	37.44	2	1	1	Calcium-transporting ATPase OS=Astyanax mexicanus PE=3 SV=1
tr|V9K9A3|V9K9A3_CALMI	37.44	2	1	1	Calcium-transporting ATPase OS=Callorhynchus milii PE=2 SV=1
tr|H2MMF7|H2MMF7_ORYLA	37.44	2	1	1	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101160819 PE=4 SV=1

tr|W5KQZ1|W5KQZ1_ASTMX	37.44	2	1	1	Calcium-transporting ATPase OS=Astyanax mexicanus PE=3 SV=1
tr|V9K7D3|V9K7D3_CALMI	36.23	2	3	1	Collagen alpha-1(I) chain OS=Callorhynchus milii PE=2 SV=1
tr|H2L470|H2L470_ORYLA	33.57	4	2	2	Uncharacterized protein OS=Oryzias latipes GN=LOC101166239 PE=3 SV=1
tr|C7ASM1|C7ASM1_SINCH	33.57	4	2	2	Muscle-type creatine kinase OS=Siniperca chuatsi PE=2 SV=1
tr|H2L804|H2L804_ORYLA	33.57	4	2	2	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101163677 PE=3 SV=1
tr|W5KP12|W5KP12_ASTMX	33.57	4	2	2	Uncharacterized protein OS=Astyanax mexicanus PE=3 SV=1
tr|W5K5Z0|W5K5Z0_ASTMX	32.92	2	3	2	Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
tr|H2LPB8|H2LPB8_ORYLA	30.14	7	2	2	Uncharacterized protein (Fragment) OS=Oryzias latipes GN=LOC101170788 PE=3 SV=1
total 137 proteins					

Collagen

Two types of collagen have been detected in both *C. striatus* water extract samples, which are type I and type II collagen. Collagen is a natural substrate for cellular attachment, growth and differentiation, and promotes cellular proliferation and differentiation. As described by Sonnemann and Bement (2011), a large amount of collagen and other extracellular matrix (ECM) proteins are secreted by the fibroblasts and myofibroblasts into the wound area, resulting in the basis of the so-called granulation tissue. Then the myofibroblasts contract the granulation tissue and the cells associated with it, closing the wound slowly and at the same time aligning the collagen fibres that composed the ECM. Many publications have shown the effect of collagen involved in the wound healing process (Kwan et al., 2011; Ruszczak, 2003). A recent study has shown that the use of concentrated collagen hydrogel favoured cell proliferation and also protected fibroblasts against apoptosis during the treatment of a chronic skin wound (Helary et al., 2012). Thus, collagen detected in the *C. striatus* sample may be another key component that assists in the wound healing process.

Uncharacterised Protein

Uncharacterised proteins consisted of more than 50% of the total proteins detected. The functions of such proteins are still unknown as the information is unavailable. However, with the high coverage detected using the PEAKS studio database matching, we are certain that the proteins exist in the sample. The uncharacterised proteins are designated as proteins of unknown functions due to no detectable homology to proteins of known functions at both the sequence and structure level (Lubec et al., 2005). Nadzirin and Firdaus-Raih (2012) has mentioned that the functions of uncharacterised proteins have not been characterised from assays following the structural analyses. The lack of information on a protein's characterised function restricts the further exploitation of these proteins. Nevertheless, there is still a possibility that one or more of the uncharacterised proteins detected in our samples are relevant to the wound healing property of *C. striatus.*

Overall, the proteome profiling using the high sensitivity LTQ-Orbitrap Velos Pro mass spectrometer has allowed us to explore the protein content in the *C. striatus* water extracts. It is evident that the extracts, which resemble local delicacy *haruan* soup, contain a considerable amount and variety of proteins. The high amount of uncharacterised proteins detected also show that the proteome database for *C. striatus* is not yet complete and requires more extensive work on the matter. Freeze-dry technique was shown to retain slightly higher amounts and variety of proteins in the water extracts than the spray-dry technique. Other parts of *C. striatus,* such the mucus layer and flesh, are currently being similarly studied. Overall, the current data has given us the insight on the proteins available in the *C. striatus* and a more detailed work has to be conducted to determine the proteins that are involved in accelerating the wound healing process.

CONFLICT OF INTEREST

The authors agree that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Soon Hong Kwan conductdt the experiment under the supervision of Mohd Nazri Ismail. Saringat Baie and Nor-

nisah Mohammed contributed with their previous experiences for solving some of the difficulties encountered during the entire project.

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REFERENCES

- Anwar SH, Kunz B (2011). The influence of drying methods on the stabilization of fish oil microcapsules: Comparison of spray granulation, spray drying, and freeze drying. J. Food Eng. 105(2): 367-378. http://dx.doi.org/10.1016/j.jfoodeng.2011.02.047
- Bach CT, Creed S, Zhong J, Mahmassani M, Schevzov G, Stehn J, Cowell LN, Naumanen P, Lappalainen P, Gunning PW (2009). Tropomyosin isoform expression regulates the transition of adhesions to determine cell speed and direction. Mol. Cell. Biol. 29(6): 1506-1514. http://dx.doi.org/10.1128/MCB.00857-08
- Baie SH, Sheikh K (2000a). The wound healing properties of Channa striatus-cetrimide cream-wound contraction and glycosaminoglycan measurement. J. Ethnopharmacol. 73(1): 15-30.
- Baie SH, Sheikh K (2000b). The wound healing properties of Channa striatus-cetrimide cream—tensile strength measurement. J. Ethnopharmacol. 71(1): 93-100.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1): 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3
- Chen C, Chi Y-J, Xu W (2012). Comparisons on the functional properties and antioxidant activity of spray-dried and freeze-dried egg white protein hydrolysate. Food Bioprocess Technol. 5(6): 2342-2352.
- Conrad PA, Giuliano KA, Fisher G, Collins K, Matsudaira PT, Taylor DL (1993). Relative distribution of actin, myosin I, and myosin II during the wound healing response of fibroblasts. J. Cell Biol. 120(6): 1381-1391. http://dx.doi.org/10.1083/jcb.120.6.1381
- Courtenay WR, Williams JD (2004). Snakeheads (Pisces, Channidae): a biological synopsis and risk assessment, US Geological Survey.
- Doherty MK, Brownridge P, Owen MA, Davies SJ, Young IS, Whitfield PD (2012). A proteomics strategy for determining the synthesis and degradation rates of individual proteins in fish. J. Proteomics. 75(14): 4471-4477. http://dx.doi.org/10.1016/j.jprot.2012.03.025
- Dominguez R, Holmes KC (2011). Actin structure and function. Annu. Rev. Biophys. 40: 169. http://dx.doi.org/10.1146/annurev-biophys-042910-155359
- Flanagan M (2000). The physiology of wound healing. J. Wound Care. 9(6): 299-300.
- Gam L-H, Leow C-Y, Baie S (2006). Proteomic analysis of snakehead fish (Channa striata) muscle tissue. Malaysian J. Biochem. Mol. Biol. 14(1): 25-32.
- Giannone G, Dubin-Thaler BJ, Rossier O, Cai Y, Chaga O, Jiang G, Beaver W, Döbereiner H-G, Freund Y, Borisy G (2007). Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell. 128(3): 561-575. http://dx.doi.org/10.1016/j.cell.2006.12.039
- Graham DM, Huang L, Robinson KR, Messerli MA (2013). Epidermal keratinocyte polarity and motility require Ca2+ influx through TRPV1. J. Cell Sci. 126(20): 4602-4613.
- Gunning PW, Schevzov G, Kee AJ, Hardeman EC (2005). Tropomyosin isoforms: Divining rods for actin cytoskeleton function. Trend. Cell Biol. 15(6): 333-341. http://dx.doi.org/10.1016/j.tcb.2005.04.007
- Helary C, Zarka M, Giraud-Guille MM (2012). Fibroblasts within concentrated collagen hydrogels favour chronic skin wound healing. J. Tissue Eng. Regen. Med. 6(3): 225-237.
- Jais AMM (2007). Pharmacognosy and pharmacology of Haruan (*Channa striatus*), a medicinal fish with wound healing properties. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 6(3): 52-60.
- Jais AMM, McCulloch R, Croft K (1994). Fatty acid and amino acid composition in haruan as a potential role in wound healing. Gen. Pharmacol. Vasc. Syst. 25(5): 947-950.
- Joshi M, Adhikari B, Aldred P, Panozzo J, Kasapis S (2011). Physicochemical and functional properties of lentil protein isolates prepared by different drying methods. Food Chem. 129(4): 1513-1522.
- Kinter M, Sherman NE (2005). Protein sequencing and identification using tandem mass spectrometry. New York, John Wiley & Sons.
- Kono K, Saeki Y, Yoshida S, Tanaka K, Pellman D (2012). Proteasomal degradation resolves competition between cell polarization and cellular wound healing. Cell. 150(1): 151-164. http://dx.doi.org/10.1016/j.cell.2012.05.030
- Kwan KH, Liu X, To MK, Yeung KW, Ho C-M, Wong KK (2011). Modulation of collagen alignment by silver nanoparticles results in better mechanical properties in wound healing. Nanomed. Nanotechnol. Biol. Med. 7(4): 497-504.
- Lees JG, Ching YW, Adams DH, Bach CT, Samuel MS, Kee AJ, Hardeman EC, Gunning P, Cowin AJ, O'Neill GM (2013). Tropomyosin regulates cell migration during skin wound healing. J. Invest. Dermatol. 133(5): 1330-1339. http://dx.doi.org/10.1038/jid.2012.489
- Lubec G, Afjehi-Sadat L, Yang J-W, John JPP (2005). Searching for hypothetical proteins: Theory and practice based upon original data and literature. Prog. Neurobiol. 77(1): 90-127.
- Maa Y-F, Nguyen P-A, Sweeney T, Shire SJ, Hsu CC (1999). Protein inhalation powders: spray drying vs spray freeze drying. Pharmaceut. Res. 16(2): 249-254. http://dx.doi.org/10.1023/A:1018828425184
- Maltesen MJ, Van De Weert M (2008). Drying methods for protein pharmaceuticals. Drug Discov. Today. Technol. 5(2): e81-e88. http://dx.doi.org/10.1016/j.ddtec.2008.11.001
- Maroto R, Hamill OP (2007). MscCa regulation of tumor cell migration and metastasis. Curr. Top. Membr. 59: 485-509. http://dx.doi.org/10.1016/S1063-5823(06)59019-2
- Martin P, Lewis J (1992). Actin cables and epidermal movement in embryonic wound healing. Nature. 360: 179-183.
- Martyniuk CJ, Denslow ND (2012). Exploring androgen-regulated pathways in teleost fish using transcriptomics and proteomics. Integr. Comp. Biol. ics072.
- Masters K (1979). Spray drying handbook. London, George Godwin Ltd.
- Matsubayashi Y, Razzell W, Martin P (2011). 'White wave'analysis of epithelial scratch wound healing reveals

how cells mobilise back from the leading edge in a myosin-II-dependent fashion." J. Cell Sci. 124(7): 1017-1021. http://dx.doi.org/10.1242/jcs.080853
- McNeil PL, Kirchhausen T (2005). An emergency response team for membrane repair. Nat. Rev. Mol. Cell Biol. 6(6): 499-505.
- Mellor JD (1978). Fundamentals of freeze-drying, Academic Press Inc.(London) Ltd.
- Messerli MA, KR Robinson (2007). MS Channels in Tip-Growing Systems. Curr. Top. Membr. 58: 393-412. http://dx.doi.org/10.1016/S1063-5823(06)58015-9
- Mohsin AM, Ambak MA (1983). Freshwater fishes of peninsular Malaysia, Universiti Pertanian Malaysia Serdang, Selangor, Malaysia.
- Nadzirin N, Firdaus-Raih M (2012). Proteins of Unknown function in the Protein Data Bank (PDB): an inventory of true uncharacterized proteins and computational tools for their analysis. Int. J. Mol. Sci. 13(10): 12761-12772. http://dx.doi.org/10.3390/ijms131012761
- Nelson G, Schmidt P, Bartolini G, Kelley D, Kyle D (1997). The effect of dietary arachidonic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids. 32(4): 421-425.
- Richardson R, Slanchev K, Kraus C, Knyphausen P, Eming S, Hammerschmidt M (2013). Adult zebrafish as a model system for cutaneous wound-healing research. J. Invest. Dermatol. 133(6): 1655-1665. http://dx.doi.org/10.1038/jid.2013.16
- Ruszczak Z (2003). Effect of collagen matrices on dermal wound healing. Adv. Drug Deliv. Rev. 55(12): 1595-1611.
- Silver M, Smith J, Ingerman C, Kocsis J (1973). Arachidonic acid-induced human platelet aggregation and prostaglandin formation. Prostaglandins. 4(6): 863-875.
- Singer AJ, Clark R (1999). Cutaneous wound healing. N. Engl. J. Med. 341(10): 738-746. http://dx.doi.org/10.1056/NEJM199909023411006
- Sonnemann KJ, Bement WM (2011). Wound repair: toward understanding and integration of single-cell and multicellular wound responses. Annu. Rev. Cell Dev. Biol. 27: 237-263. http://dx.doi.org/10.1146/annurev-cellbio-092910-154251
- Wee KL (1982). Snakeheads–their biology and culture. Recent Adv. Aquacult. 1: 179-213.
- Witkowski C, J Harkins (2009). Using the GELFREE 8100 Fractionation System for molecular weight-based fractionation with liquid phase recovery. J. Visualized Exp. e1842.
- Witte MB, Barbul A (1997). General principles of wound healing. Surg. Clin. North Am. 77(3): 509-528. http://dx.doi.org/10.1016/S0039-6109(05)70566-1
- Zheng JQ, Poo M-m (2007). Calcium signaling in neuronal motility. Annu. Rev. Cell Dev. Biol. 23: 375-404. http://dx.doi.org/10.1146/annurev.cellbio.23.090506.123221