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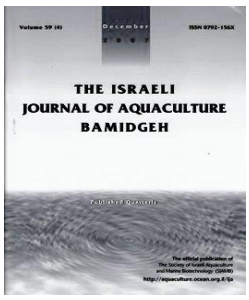

A Comparative Study of Survival, Metabolism, Immune Indicators, and Proteomics, in Five Batches of Japanese Scallop *Mizuhopecten yessoensis* under Short-Term High Temperature Stress

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Key words: *Mizuhopecten yessoensis*, heat resistance, survival rate, oxygen consumption rate, immune indicators, proteomics, high temperature

Abstract

Five batches of the Japanese scallop *Mizuhopecten pyessoensis* were tested for survival rate, oxygen consumption, catalase (CAT) and superoxide dismutase (SOD) activities, total antioxidant capacities (T-AOC) contents, and proteomics under short-term high temperature conditions. The five batches, (W1, W2, W3, W4, W5) selected from the established 21 'ivory white' *M. yessoensis* batches, had higher survival rates than the other batches after one year of culture. Initial rearing water temperature of 15°C was increased by 1°C per day with a cooling and heating system. The temperature was raised until over 50% of the scallops from 3 batches died. This occurred at 30°C. The higher than normal culture temperature conditions showed significant or highly significant differences in the responses of some of the batches. Some showed significantly higher survival rates and significantly different rates of oxygen consumption. CAT activity, SOD activity and T-AOC content was similar in the five batches, and all three indices were significantly lower in W3 and W5 than in the other batches ($P<0.01$). Expression patterns of MDA content were opposite to those of CAT activity, SOD activity and T-AOC content. Protein profiles of all five batches were similar; the sizes of the predominant bands ranged from 20-110 kDa. We identified twenty-eight proteins with high scores in the database. These included heat shock proteins (HSPs), glucose-regulated protein 94, and arginine kinase.

In conclusion, survival, CAT and SOD activities, T-AOC, MDA content and proteomics in the W1, W2, and W4 batches responded favorably to elevated culture temperatures. These batches may be advantageous in selecting a high-temperature-resistant strain of *M. yessoensis*.

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Introduction

The Japanese scallop, *Mizuhopecten yessoensis*, is a cold-water shellfish that lives in water with temperatures ranging between 0-23ºC. It is widely distributed along the coastlines of the northern islands of Japan, the northern region of the Korean Peninsula, and the Sakhalin and Kuril Islands. It was introduced from Japan into China in the 1980s (Ding *et al* 2011). Commercial culture of *M. yessoensis* has rapidly expanded, and the Japanese scallop has become an economically important aquaculture species along the northern coastline of China, as it is larger and commands a higher market price than both the native scallop, *Chlamys farreri* and the introduced bay scallop, *Argopecten irradians.* In China, two cultivation methods are used: suspended culture and bottom culture (Ding *et al* 2011).

In recent years, *M. yessoensis* stock in northern China has seriously depleted. From July to August each year, large quantities of 1-year-old *M. yessoensis* in suspended culture die. This has seriously affected the *M. yessoensis* industry resulting in major losses for local fishermen (Li and Tan 2009). The observed stock decline may have been caused by high water temperatures in the summer induced by global warming, which exceeded the scallops' tolerance. Shellfish have limited thermoregulation ability, and their body temperature is primarily dependent on ambient temperatures. Thus, changes in ambient temperature could have affected the physiological condition of shellfish (Fearman and Moltschaniwskyj 2010). Genetic degradation of *M. yessoensis* may also be a contributory factor in stock decline.

A small number of *M. yessoensis* was introduced to China and this could have lead to poor performance in growth, size, and resistance to environmental stresses after multiple cycles of artificial breeding and selection by producers. It is therefore essential to study the genetics of *M. yessoensis* and develop varieties that can overcome these problems (Zhang et al., 2014).

Our research team bred 'ivory white' strains of *M. yessoensis* after long-term selective breeding. Both the left and right shells are white in these strains, whereas in unselected individuals the left shell is brown and the right shell is a creamy yellow color. Relative to common *M. yessoensis*, 'ivory white' strains have been shown to exhibit good performance in suspended culture in terms of growth, dressing percentage, and resistance to environmental stress, as well as significantly higher tolerance to high temperatures. However, there has been relatively little scientific data reported to support these observations until now.

In this study, we analyzed the survival rates, oxygen consumption, catalase (CAT) and superoxide dismutase (SOD) activities, total antioxidant capacities (T-AOC), malondialdehyde (MDA) contents, and proteomics of five 'ivory white' *M. yessoensis* batches (W1–W5) at high temperatures. The results of this study may provide data to support the screening of *M. yessoensis* for resistance to higher temperatures.

Materials and Methods

Family source. M. yessoensis individuals used to establish the batches for this study were second generation 'ivory white' individuals cultivated by our research team since 2009. The batches with the largest numbers of individuals were allowed to mature under artificially warm conditions to generate a third 'ivory white' strain in March 2011. The selected individuals (46 females and 46 males) were then used to produce a third-generation. The fertilized eggs produced planktonic larvae after approximately 72 h, in water of 10-13°C. A total of 25 *M. yessoensis* 'ivory white' batches were established after this selection procedure. The planktonic larvae metamorphosed into larvae after approximately 20 days. Two weeks later they were transferred to the local bay for short-term culture. After 21 'ivory white' *M. yessoensis* batches were successfully established five batches (W1, W2, W3, W4 and W5) which had higher survival rates than the other batches after one year of culture, were selected .

Experimental system. The high-temperature experiments were conducted in April 2012 at the Key Laboratory of North Mariculture, Agriculture Ministry, Dalian

Ocean University, Liaoning, China. Five 'ivory white' strain batches of *M. yessoensis* scallops (450 scallops, 90 scallops per family) were randomly assigned to and acclimated in 15 square glass tanks (70 × 40 × 40 cm). Each family was cultured in 3 tanks (replicates) with 30 scallops per tank, each tank with an effective water volume of 75 L. These were acclimated for 2 weeks prior to onset of experiments. They were fed twice daily (09:00 and 20:00 h) with Sargassum and Spirulina powder. The tank water was maintained at 15 ± 0.5 °C using a cooling and a heating system. The tanks maintained a salinity of 30.00 ± 1.00g · L−1, a day/night photoperiod of 14 D/10 N, and a pH of 7.50 ± 0.07. Water was exchanged at a daily rate of 30% of the tank volume using 1 μm filtered and ultraviolet-sterilized seawater.

Temperature changes. After 2 weeks of acclimation, 20 scallops from each tank were randomly selected for experimentation. Water temperature was then increased by 1°C per day. The temperature increase continued until 50% scallops in all 3 batches had died; this occurred at 30°C. Next, we randomly selected 1 surviving scallop from each replicate and collected the coelomic fluid from the adductor muscle with a 5 mL syringe. This fluid was then immediately transferred into 2 mL centrifuge tubes and placed in an ice bath. The tubes of coelomic fluid were then centrifuged for 10 min at 4,500 rpm, and the resulting supernatants were stored at −80 ºC for assays of CAT and SOD activity, T-AOC, MDA content, and proteomics. Scallops were considered to be dead if they did not move or respond to touch. Dead scallops were removed at each observation. Within 2 h of feeding, any remaining food and feces were siphoned out of the tanks.

Oxygen consumption. At the end of the experiment, when over 50% of the scallops in the 3 batches had died, one surviving scallop from each replicate was transferred to a 2,000 ml volumetric flask containing seawater to measure oxygen consumption. The flasks were sealed and placed in a water bath at 30°C for 3 h. The DO concentration was > 4.0 mg L−1 at the end of the experiment. Water samples were collected by siphoning. DO of the water samples was measured at the beginning and end of the experiment with the Winkler method (Chen 2000). DO readings were recorded from control samples (without scallops). These values were subtracted from the experimental data to correct for autogenic trends. The mean differences in DO between the beginning and the end were calculated (mg/g/h) and the wet weight and duration of exposure (h) of the scallops in each flask was determined.

CAT, SOD, T-AOC, and MDA analyses. The assays of CAT and SOD activity, T-AOC, and MDA content were conducted using commercial kits (Jiancheng, Nanjing, China). CAT levels were calculated based on the ability of catalase to decompose hydrogen peroxide, which strongly absorbs light at 240 nm; the reactive solution absorbance (A240) therefore decreases with time. SOD assays were based on the ability of the xanthine-xanthine oxidase system to inhibit the oxidation of oxyamine. T-AOC levels were calculated based on the ability of the antioxidants within the samples to reduce ferric ion (Fe3+) to a ferrous chelates (Fe2+), which absorb light at 520 nm. MDA reacts with thiobarbituric acid (TBA) to produce a red complex with peak absorbance at 532 nm according to Buege and Aust (1984). The CAT and SOD activities and T-AOC and MDA contents were measured in U/mL.

SDS-PAGE and Gel analysis. The coelomic fluid was centrifuged for 10 min at 4,500 rpm, and a small amount of supernatant was used to determine protein concentrations according to the Bradford method (Bradford,1976) Briefly, 160μL supernatant plus 40μL 5× buffer (250 mM Tris-HCl pH 6.8, 10%w/v sodium dodecyl sulfate, 0.5%w/v BPB, 50%v/v glycerol and 5%w/v β-mercaptoethanol) was incubated at 100°C for 10 min, followed by centrifugation at 12,000 rpm for 20 min. The remainder was stored at −20°C until analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% separating gel, 6% concentrated gel). The gel was stained with silver as described previously (Guo,1999) and then scanned using a CanoScan 9000F image scanner (Cano Company, Thailand) for image and data analyses. Protein bands of interest were excised from silver-stained gels and washed twice with distilled water. They were

then sent to Beijing Genomics Institute (Wuhan City, Hubei Province, China) for protein mass spectrometry and identification of differentially expressed proteins.

Enzymatic in-gel digestion. The differentially expressed protein bands were sent to Beijing Genomics Institute and cut into small pieces. The gel pieces were washed in equal volumes of 100% methanol and 200 mM ammonium bicarbonate for 15 min at least twice to remove the CBB G-250. The gel pieces were then dehydrated with 100% ACN for 5 min, vacuum dried and rehydrated with 250ng of sequencing-grade, modified trypsin (Promega, Madison,WI, USA) in 50 mM ammonium bicarbonate, pH 8.5, at 37^0 for 16 h. The digested samples were collected and vacuum dried. Samples were dissolved in 50% v/v ACN formic acid and subjected to LC-MS/MS.

LC-MS/MS and Database searching. Trypsin digestion sample of 20 uL was loaded onto a BioBasic-18 column, 100×0.18mm, particle Sz. (5u) (Prominence nano, SHIMADZU, Japan) and separated. The column was connected directly to the Q-EXACTIVE (Thermo Fisher Scientific, San Jose, CA).
The MS/MS spectra search against Mollusca_NCBI (84275seqs) was done using Mascot2.3.02.
(http://www.ncbi.nlm.nih.gov/protein/?term=txid6447[Organism:exp]

Proteomics SDS-PAGE protein profile. The coelomic fluid proteins of the five *M. yessoensis* batches are shown in Fig 1. The protein profiles of all five batches were similar, and the sizes of the predominant bands were estimated to range from 20–110 kDa. Three strongly up-regulated bands (a), (b) and (c) located in lanes 1, 2, and 4; Fig. 1) were selected and sent to BGI for protein mass spectrometry analysis.

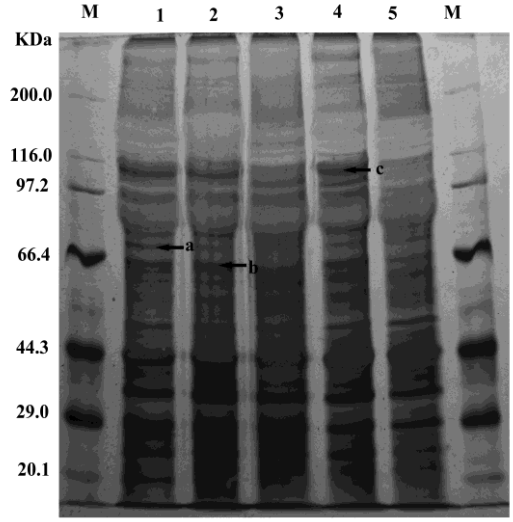

Fig.1. SDS-PAGE analysis of coelomic fluid proteins from five batches of *M. yessoensis*. Lane M: protein molecular mass markers in kDa; lanes 1–5: proteins from pedigrees W1, W2, W3, W4 and W5; a, b and c: protein strips for subsequent analysis.

Data analysis. Differences in the survival rate, oxygen consumption rate, CAT and SOD activities, T-AOC, and MDA content among treatments were analyzed using one-way analyses of variance (ANOVA). Before analysis, percentage data were arcsine square-root transformed. When ANOVA indicated a significant difference ($P< 0.05$) among treatment levels, Duncan's multiple range tests were used to identify significant differences between different treatment levels. The relationship between temperature and survival was estimated using least square regression, and the significance was tested using ANOVA. These statistical analyses were performed using SPSS 13.0 statistical software, and the significance level was $P< 0.05$ for all analyses.

Results

Survival rate. The survival rates differed significantly among the batches at 30 °C (one-way ANOVA, $F (4, 10) = 12.817$, $P = 0.001$) (Fig. 2). The lowest survival rates were 20.0% and 21.67% in batches W3 and W5, respectively, with no significant difference between them ($P>0.05$). The highest survival rate was 63.33% in W1, which was significantly higher than those of W3 and W5 ($P<0.01$)

and slightly higher than those of W2 and W4 (P>0.05). The survival rates of W2 and W4 were 50.17% and 48.33%, respectively.

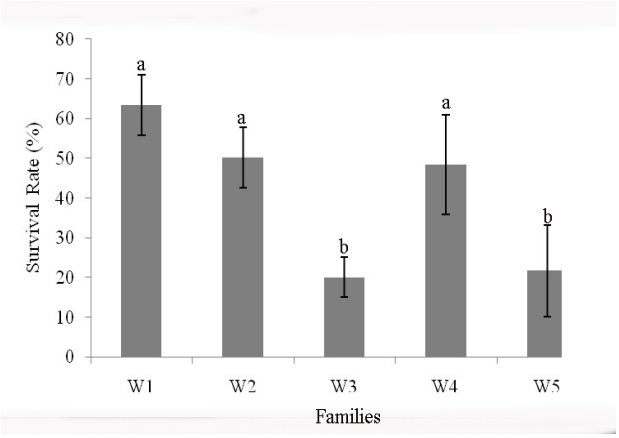

Fig. 2. Survival rates of five batches of *M. yessoensis* exposed to 30°C. Bars represent mean values and error bars represent the standard errors of the means. Different letters above the error bars indicate significant differences (*P*< 0.05).

Metabolism. The oxygen consumption of juveniles at 30°C differed among the five batches of *M. yessoensis* (one-way ANOVA, F (4, 10) = 10.936, P = 0.001) (Fig. 3). Duncan's multiple range tests showed highly significantly lower oxygen consumption in W3 and W5 and highly significantly greater in W1 (P<0.01) than in the other batches. However, no significant differences (P>0.05) in oxygen consumption were found between the W3 and W5 batches or among the W1, W2 and W4 batches.

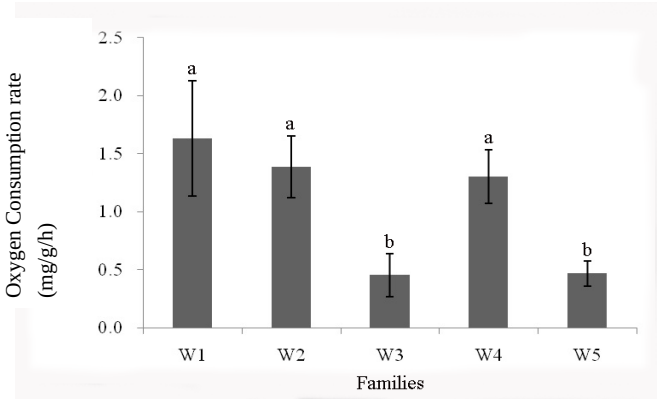

Fig. 3. Oxygen consumption rates in five batches of *M. yessoensis* exposed to 30°C. Bars indicate the mean values (n=3) and error bars represent the standard errors of the means. Different letters above the error bars indicate significant differences (*P*< 0.05).

CAT and SOD activities, T-AOC, and MDA content. Figure 4 shows the CAT and SOD activities, T-AOC, and MDA contents of scallops from the five different batches at the tested temperature. Patterns of CAT activity, SOD activity and T-AOC content were similar among the five batches, and all three indices were highly significantly lower in W3 and W5 than in the other batches (P < 0.01). There were no significant differences between W3 and W5 in any of these parameters, whereas the parameter values of W1 were significantly higher than those of W3 and W5 (P<0.01) and slightly higher than those of W2 and W4; however, the differences among the W1, W2 and W4 batches were not significant (P> 0.05). Opposite expression patterns were revealed for MDA content as compared to those for CAT activity, SOD activity and T-AOC content. The MDA activities in W3 and W5 were highly significantly greater than those in W1, W2, and W4 (P < 0.01). However, there were no significant differences between W3 and W5 or among W1, W2, and W4 (P > 0.05).

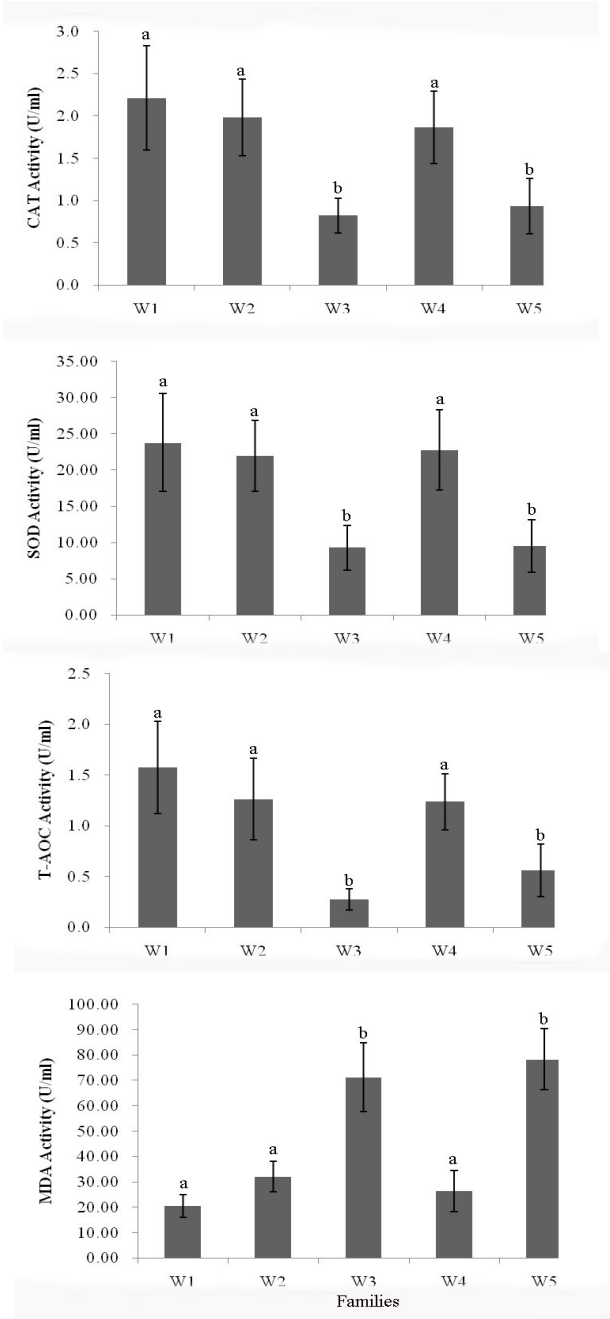

Fig. 4. CAT and SOD activities, T-AOC, and MDA content in five batches of *M. yessoensis* at 30°C. Bars represent the mean values (n=3) and error bars represent the standard errors of the means. Different letters above the error bars indicate significant differences ($P< 0.05$).

Protein identification. The identified proteins were annotated by BLAST searches against the Mollusca NCBI (84,275seqs) database and classified into several groups according to their functions (Tables 1, 2, and 3).The identified proteins from the three strips were assigned to categories according to their involvement in biological processes, cellular components, and molecular functions. For clarity and reliability, the classification was based on Gene Ontology classification (http://www.geneontology.org) using the Protein Information Resource (http://pir.georgetown.edu/).

Table1. List of identified proteins from strip a by MS/MS

NO.	Score	Coverage (%)	Protein name	Accession number	Mr(KDa)/*p*I
Proteins related to stress					
1	2245	41.4	Heat shock protein 70 [*Mizuhopecten yessoensis*]	gi|42494889|gb|AAS17724.1|	71/5.1
2	1072	21.2	Heat shock protein 90 [*Azumapecten farreri*]	gi|38146757|gb|AAR11781.1|	83/4.5
3	529	10.7	Heat shock protein 70 [*Mytilus galloprovincialis*]	gi|76780610|emb|CAH04108.1	69/5.1
4	496	10.4	Heat shock inducible protein70 [*Haliotis diversicolor*]	gi|225906409|gb|ACO36048.1|	70/5.2
5	300	26.5	Heat shock protein 70 [*Modiolus modiolus*]	gi|158563805|gb|ABW74356.1	18/4.5
6	200	3.5	Glucose-regulated protein 94 [*Crassostrea gigas*]	gi|148717303|dbj|BAF63637.1|	91/4.5
7	141	6.6	Calcium-ATPase [*Mizuhopecten yessoensis*]	gi|4165020|dbj|BAA37143.1|	111/5.0
8	61	5.4	Heat shock protein 70 [*Aplysia californica*]	gi|2706557|emb|CAA78756.1|	24/7.5
9	55	2.4	Stress-70 protein mitochondrial [*Crassostrea gigas*]	gi|405950030|gb|EKC18038.1|	76/5.6
Proteins involved in					
10	64	2.9	Glycogen phosphorylase [*Crassostrea gigas*]	gi|50512854|gb|AAS93901.1|	99/6.3
11	63	2.5	Dipeptidyl-peptidase 3 [*Crassostrea gigas*]	gi|405966068|gb|EKC31390.1|	84/5.5
12	62	3.3	Arginine kinase [*Azumapecten farreri*]	gi|366091039|gb|AEX08673.1|	41/7.1

Table2. List of identified proteins from strip b by MS/MS

NO.	Score	coverage(%)	Protein name	Accession number	Mr(KDa)/*p*I
Proteins related to stress					
1	1137	37.4	Heat shock protein 70 [*Mizuhopecten yessoensis*]	gi|42494889|gb|AAS17724.1|	71/5.1
2	430	8.9	Heat shock protein 90 [*Azumapecten farreri*]	gi|38146757|gb|AAR11781.1|	83/4.5
3	221	3.5	Glucose-regulated protein 94 [*Crassostrea gigas*]	gi|148717303|dbj|BAF63637.1|	92/4.5
4	216	14	Heat shock protein 70 [*Dreissena polymorpha*]	gi|145617263|gb|ABP88104.1|	34/6.5
5	203	6.7	Heat shock protein 70 [*Mytilus galloprovincialis*]	gi|62989584|emb|CAE51348.1|	70/5.1
6	139	6.3	Calcium-ATPase [*Mizuhopecten yessoensis*]	gi|4165020|dbj|BAA37143.1|	111/5.0
7	131	26.5	Heat shock protein 70 [*Modiolus modiolus*]	gi|158563805|gb|ABW74356.1|	18/4.5
Proteins involved in immunity					
8	141	31	Tropomyosin [*Mizuhopecten yessoensis*]	gi|2196554|dbj|BAA20455.1|	32/4.3
9	69	2.6	Ubiquitin-like modifier-activating enzyme 1	gi|405958211|gb|EKC24358.1|	60/6.9
Proteins involved in metabolism					
10	1499	25.5	Endoglucanase [*Mizuhopecten yessoensis*]	gi|254553092|dbj|BAH85844.1|	65/5.9
11	231	7.3	Pyruvate kinase [*Crassostrea gigas*]	gi|113207856|emb|CAJ28914.1|	62/6.9
12	90	2.9	Glycogen phosphorylase [*Crassostrea gigas*]	gi|50512854|gb|AAS93901.1|	99/6.3
13	88	1.4	Alpha macroglobulin [*Azumapecten farreri*]	gi|40074252|gb|AAR39412.1|	196/4.7
14	70	3.3	Arginine kinase [*Azumapecten farreri*]	gi|366091039|gb|AEX08673.1|	41/7.1

Table3. List of identified proteins from strip c by MS/MS

NO.	Score	coverage(%)	Protein name	Accession number	Mr(KDa)/*p*I
Proteins related to stress					
1	632	12.7	Calcium-ATPase [*Mizuhopecten yessoensis*]	gi|4165020|dbj|BAA37143.1|	111/5.0
2	54	3.7	Heat shock protein 90 [*Azumapecten farreri*]	gi|38146757|gb|AAR11781.1|	83/4.5
Proteins involved in immunity					
3	1478	5.1	Scavenger receptor cysteine-rich protein	gi|238821196|gb|ACR58424.1|	88/4.4
4	56	1.4	Lupus brain antigen 1-like protein	gi|405959227|gb|EKC25284.1|	98/5.3
Proteins involved in metabolism					
5	108	3.3	Arginine kinase *[Azumapecten farreri*]	gi|366091039|gb|AEX08673.1|	41/7.1
6	80	1.5	Low-density lipoprotein receptor-related protein	gi|405960567|gb|EKC26481.1|	76/4.7

Discussion

In the present study, genetic selection was conducted using family selection with individuals selected according to their relative family traits at the end of the experiment. Pedigree selection can be applied to some low-heritability traits, such as fecundity, survival rate, disease resistance and fast breeding (He *et al*, 2008). This method was further demonstrated in experiments reported by He *et al* (2007) and Chen *et al* (2008). He *et al* (2007) established seven *Pinctada martensi* batches that showed good growth performance, and Chen *et al* (2008) selected four fast-growing batches and three disease-resistant pedigrees from among 63 established *Paralichthys olivaceus* pedigrees.

Some researchers suggested that shell color could be used as a selectable trait and that the relationships between shell color and phenotypic traits may be associated with genetic and physiological characteristics of the shellfish and with ecological conditions (Zheng *et al*, 2003). Our results confirm this viewpoint and showed that batches of *M. yessoensis* W1, W2 and W4 exhibited good survival performances when water temperature reached 30ºC with survival rates 63.33%, 50.17% and 48.33%, respectively. These survival rates were much higher than Hao *et al*. (2014) reported. Hao *et al* (2014) found that the survival rates of dark shell colored *M. yessoensis* was only 25.89 % when temperature was 26ºC. Similar results also appeared in the research of Zhang *et al* (2003) research. Zhang *et al* (2003) found that white-shelled *Chlamys farreri* scallops had higher survival rates than darker shelled ones while lighter shell colored individuals had higher survival rates compared with darker shelled individuals at high temperatures. Lighter shell colored scallops absorb less radiant energy than darker shelled ones (Mitton, 1977). The absorption of less heat energy from the sun would avoid overheating and mortality. However, further studies are needed to clarify this relationship.

Increasing evidence suggests that exposure to environmental stress can induce the generation of reactive oxygen species (ROS), such as superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxide radicals (OH^-), and singlet oxygen ($^1O_2$) (Zhang *et al* 2008). Higher rates of ROS may have detrimental effects on metabolism, initiate reaction cascades that result in the production of toxic chemical species and ending in cell dysfunction and death as found in *Mya Arenaria* (Greco *et al* 2011). CAT and SOD appear to play an essential protective role in the ROS scavenging process. SOD converts O_2^-radicals into H_2O_2 and O_2, and CAT can dismutase H_2O_2 into H_2O and O_2. Moreover, the levels of MDA, a product of lipid peroxidation, are often used as markers of oxidative stress (Greco *et al* 2011). For *M. yessoensis*, temperature is one of the most important environmental factors affecting physical activity, thus temperature changes may directly affect the immune defense ability of this species.

The results of our experiments suggest that CAT and SOD activities, T-AOC, and MDA contents at a water temperature of 30°C varied significantly among the five batches of *M. yessoensis*, with the values for CAT, SOD activity, and T-AOC all being highly significantly lower in W3 and W5 than in W1, W2 and W4 ($P < 0.01$). The relative patterns of MDA content were opposite to those of CAT

activity, SOD activity and T-AOC content. These results indicate that W1, W2, and W4 were less affected by a temperature of 30°C, whereas W3 and W5 were less tolerant to this temperature. This could suggest an overproduction of reactive oxygen species and reduced antioxidant enzymes and increased risk of oxidative damage. Oxidative damage in turn leads to cell death, a process that could explain the high mortality recorded for the W3 and W5 batches.

Some authors suggest that protein synthesis and decomposition act as countermeasures for resisting environmental stress (Lovett *et al* 2001). In this study, we selected three protein bands that were differentially expressed among the five batches of *M. yessoensis* at high temperatures. We identified 28 proteins related to our research that showed high scores in the database. These 28 proteins included heat shock proteins (HSPs), glucose-regulated protein 94 (GRP94), and arginine kinase. There were 12 HSPs (HSP90s and HSP70s), accounting for the largest proportion of the 28 identified proteins. These proteins could potentially act as molecular markers for high-temperature resistance in *M. yessoensis*.

HSPs comprise a protein family that can act as molecular chaperones to promote the renaturation of denatured proteins and the folding of newly-synthesized proteins. HSPs are also involved in protein secretion and degradation (Sarkar *et al* 2011). Zou *et al* (2011) found that HSPs accumulated in organisms exposed to thermal stress, thus increasing the organism's ability to resist high temperatures. The results of our present study support those of Zou *et al* (2011) by showing that HSPs were more abundant in W1, W2, and W4 than in the other two batches. This suggests that the accumulation of HSPs in *M. yessoensis* may be related to increased survival rates observed at high temperatures.

Glucose-regulated protein 94 (GRP94) is also a molecular chaperone and stress protein, the expression of which increases when cells are exposed to adverse factors, thereby contributing to the organism's immune response. Glucose-regulated protein 94 is an important part of the HSP family, although its immune function in aquatic animals has not yet been reported (Lee 2001). HSP70A, HSP70B and GRP78 appear to show increased expression levels in response to temperature after an acute 2-h heat shock experiment, in *Laternula elliptica* and *Nacella concinna (*Melody *et al*., 2008). In the present study, glucose-regulated protein 94 levels increased in the W1, W2, and W4 batches after high-temperature stimulation, implying that it may be involved in the ability of *M. yessoensis* to resist high temperatures. However, further research is needed to address this issue.

Arginine kinase is widely found in invertebrates and is one of the most important enzymes associated with energy metabolism. Its main function is to provide energy for normal metabolic activities and maintain the ATP balance of the organism. However, Su *et al* (2011) suggested that arginine kinase was not only directly related to energy metabolism but also involved in the body's resistance and immunity under stress conditions. Zhou *et al* (2010) also found arginine kinase in differentially expressed proteins that were identified by LC-MS/MS in larvae of *Artemia sinica* exposed to 5 mmol/L copper sulfate for 24 h. In accordance with these results, arginine kinase expression levels in the present study were higher in the W1, W2, and W4 *M. yessoensis* batches than in the W3 and W5 batches after high-temperature stimulation. This suggests that arginine kinase may play a role in the ability of *M. yessoensis* to resist high temperatures. However, further studies are needed to confirm these results.

In conclusion, short-term exposure to high temperatures stimulated the expression of heat-response proteins in the W1, W2, and W4 batches of *M. yessoensis* but not the W3 and W5 batches, indicating that *M. yessoensis* may synthesize these proteins as a short-term adaptation tool. If so, it might be possible to select *M. yessoensis* individuals that show long-term genetic adaptation to high temperatures. The comparative proteomics of *M. yessoensis* under long-term high temperatures thus warrant further research. The W1, W2, and W4 batches exhibited good survival performance as well as CAT and SOD activities, T-AOC, MDA contents and proteomics in response to high

temperatures. We believe that these batches represent a good basis for further selection, with the aim of developing a high-temperature-resistant strain of *M. yessoensis*.

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References

Bradford M.M., 1976. *Anal Biochem* 72: 248-254.

Buege J.A. and S.D. Aust,1984. Microsomal lipid peroxidation. *Methods in Enzymology* 105: 302–310.

Chang Y.Q., 2007. Mollusc Culture and Enhancement. Beijing: *China Agricultural Press*. 91-97 (In Chinese).

Chen J.R., 2000. A practical handbook of seawater analysis. Beijing: *China Agriculture Press*, pp 100-153.

Chen S.L., Tian Y.S., Xu T.J., Deng H., Liu S.T., Liu B.W., Ji X.S. and G.C. Yu, 2008. Development and characterization for growth rate and disease resistance of disease-resistance population and family in Japanese flounder (Paralichthys olivaceus). *J Fish China* 32 (5): 665-673. (In Chinese with English abstract).

Ding J., Chang Y.Q., Zhang J. and Z.L. Hao, 2011. Analysis and evaluation of nutritive composition in " Ivory " strains and common strains of Mizuhopecten yessoensis. *J Agric Sci Technol* 13(2): 121-128. (In Chinese with English abstract).

Fearman J. and N.A. Moltschaniwskyj, 2010. Warmer temperatures reduce rates of gametogenesis in temperate mussels, Mytilus galloprovincialis. *Aquaculture* 305(1): 20-25.

Greco L., Pellerin J., Capri E., Garnerot F., Louis S., Fournier M., Sacchi A., Fusi M., Lapointe D. and P. Couture, 2011. Physiological effects of temperature and a herbicide mixture on the soft-shell clam Mya Arenaria (*Mollusca, Bivalvia*). *Environ Toxicol and Chem* 30: 132–141.

Guo Y.J., 1999. Protein electrophoresis technology. Beijing: *Science Press*, pp. 142. (In Chinese).

Hao Z.L., Tang X.J., Ding J., Ben Y. and Y.Q. Chang, 2014. Effect of high temperature on survival, oxygen consumption, behavior, ammonia-N excretion, and related immune indicators of the Japanese scallop Mizuhopecten yessoensis. *Aquacult Int* 22:1863–1876.

He M.X., Guan Y.Y., Lin Y.G. and L.M. Huang, 2007. Growth comparison between families of pearl oyster Pinctada martensii Dunker. *J tropical oceanogr* 26 (1): 39-43. (In Chinese with English abstract).

He Y.Y., Li J., Liu P., Huang F.Y. and Q.Y. Wang, 2008. Comparison of the resistance to pH value and ammonia in Chinese shrimp (*Fenneropenaeus chinensis*) families. *Periodical of Ocean University of China* 38(5): 761-765. (In Chinese with English abstract).

Lee A.S., 2001. The glucose-regulated proteins stress induction and clinical applications. *Trends in Biochem Sci* 8(26): 504-510.

Li W.J., Tan K.F, 2009. The Inspiration on massive mortality of scallop Patinopecten yessoensis resolved by Japan. *Fisheries Science* 10: 609-612. (In Chinese with English abstract).
Lovett D.L., Verzi M.P., Clifford P.D. and D.W. Borst, 2001. Hemolymph levels of methyl farnesoate increase in response to osmotic stress in the green crab, Carcinus maenas. *Comp Biochem Physiol A: Mol Integr Physiol* 128: 299-306.
Melody S., Keiron P. and S. Lloyd, 2008. Antarctic marine molluscs do have an HSP70 heat shock response. *Cell Stress and Chaperones* 13:39–49.
Mitton J.B., 1977. Shell color and pattern variation in Mytilus edulis and its adaptive significance. *Chesapeake Sci* 18(4): 387-390.
Sarkar S., Singhl M.D., Yadav R., Arunkumar K.P. and G.M. Pittman, 2011. Heat shock proteins: molecules with assorted functions. *Front Biol* 6: 312–327.
Su X., Lu G. and H. Cheng, 2011. Progress in the studies on protein and biology of arginine kinase. *Biotech Bull* 4, 26-30.
Yokoyama Y., Hashimoto H., Kubota S., Kuriyama A., Ogura Y., Mizuta S., Yoshinaka R. and H. Toyohara, 2006. cDNA cloning of Japanese oyster stress protein homologous to the mammalian 78-kDa glucose regulated protein and its induction by heatshock. *Fish Sci* 72:402–409.
Zhang g.f., liu S.X., Liu X., Guo X.M. and F.S. Zhang, 2003. Self-fertilization family establishment and its depression in bay scallop Argopecten irradians. *J Fish Sci China* 10(6): 441-445. (In Chinese with English abstract).
Zhang S., Li Li., Zhang G., 2014. Using Microsatellites to Assess Genetic Variation in a Selective Breeding Program of Chinese Bay Scallop (*Argopecten irradians irradians*), 9 pages. *Isr. J. Aquacult. - Bamidgeh,* [I*JA_65.2013.933*]
Zhang Y., Tang H.R. and Y. Luo, 2008. Variation in antioxidant enzyme activities of two strawberry cultivars with short-term low temperature stress. *J Agr Sci* 4(4): 458-462.
Zheng H.P., Zhang G.F., Liu X. and H.Y. Que, 2003. Establishment of different shell color lines of bay Scallop argopecten irradians Lamarck (1819) and their development. *Oceanologia Et Limnologia Sinica* 36(6): 632-639. (In Chinese with English abstract).
Zhou Q., Wu C.G., Dong B., Li F.H., Liu F.Q. and J.H. Xiang, 2010. Proteomic analysis of acute responses to copper sulfate stress in larvae of the brine shrimp, Artemia sinica. *Chinese J Oceanol Limnol* 28(2), 224-232.
Zou J., Liu C. and X. Chen, 2011. Proteomics of rice in response to heat stress and advances in genetic engineering for heat tolerance in rice. *Plant Cell Rep* 30: 2155–2165.