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Feeding Artemia larvae with yeast heat shock proteins 82 (HSPs82) to enhance the resistance against abiotic stresses (hyperosmotic and high temperatures)

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Abstract

Feeding farmed Artemia with yeast heat shock proteins is a novel way to protect them from stress conditions during the culture. In this study, the effect of feeding with stressed new identified Saccharomyces cerevisiae strain YG3-1 yeasts (containing induced heat shock proteins) on the survival of Artemia in stress conditions, was evaluated. For this purpose, heat shock proteins 82 (Hsps 82) of mentioned yeasts were induced by applying the high thermal (30, 35 and 40 °C) and high salinity (60, 120, 180 and 240 g.l⁻¹) stresses. After that, two different species of Artemia (Artemia urmiana and A. franciscana) were fed with treated yeasts during the culture. Then, to investigate the effects of S. cerevisiae strain YG3-1 Hsps82 on Artemia survival, after the end of feeding, adult individuals of both species were exposed to authorized high salinity (230 g L⁻¹ and 280 g L⁻¹) and authorized high temperature (35 °C and 37 °C) as permitted stress for 48 h (hours). Finally, this administration resulted in the resistance of both species against the high salinity and high temperature (p < 0.05). This result was confirmed by analysing total protein of Artemia using SDS-PAGE, and suggests that this administration can be used for enhancing the survival of Artemia in stress conditions.

Keywords: Artemia, Heat shock proteins, Saccharomyces cerevisiae, Stress, Survival

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Introduction

Artemia is one of the most important live foods in aquaculture (Garcia et al., 2008), and is favored as a model use in aquaculture organism for biotechnology (Gavanda et al., 2007). Heat shock proteins (Hsps) are a large class of proteins (Tkáčová Angelovičová, 2012), and play a key the process of protein role metabolism under normal and stress conditions, including the refolding of denatured protein, maintenance of structure integrity and other regulatory processes (Qin et al., 2016). An increased accumulation of Hsps is essential for the survival of cells exposed to various stresses (Li et al., 2006). There are multiple stressors in a changing world (Gunderson et al., 2016). For example, the effects of global warming include rising mean annual temperatures and increase in the frequency and amplitude of severe temperature events (Xu et al., 2016). So, farmed aquatic organisms experience different many environmental including stresses temperature fluctuation and salinity shift during culture (Aleng et al., 2015). The production of heat shock proteins is one of the classical cellular responses of all organisms to environmental insult (Clark et al., 2008). The enhanced expression of heat shock proteins in aquatic organisms can be detected in response to many kinds of the stressor (Shi et al., 2015). Heat shock proteins are a kind of resistance mechanisms against environmental stresses such as hyperosmotic and high temperatures in Artemia and other aquatic crustaceans

(Sankian et al., 2011). Enhancement of Hsps synthesis promotes resistance of aquatic organisms against conditions (Sung et al., 2012). Heat shock proteins can be produced in these organisms by exposure to stress (Sung et al., 2008; Givskov Sorensen, 2010), and also through feeding with single cell organisms (Probiotics) containing induced Hsps (Sung et al., 2009a). Examples include observations Hsp70-induced thermotolerance generated in common carp (Cyprinus carpio L.,) against lethal ammonia toxicity and in coho salmon (Oncorhynchus kisutch) as result of a sublethal heat shock (Sung et al., 2012; Arkush et al., 2008). In another study, feeding with bacterial heat shock proteins protected Artemia franciscana larvae from Vibrio campbellii infection (Sung et al., 2009a). Feeding of bacterially encapsulated heat shock proteins to invertebrates is a novel way to limit Vibrio infection. As example, ingestion of Escherichia coli overproducing prokaryotic Hsps significantly improves survival gnotobiotically cultured Artemia larvae upon challenge with pathogenic V. campbellii (Sung etal.. 2009b). Considering that in recent years researchers have investigated that the proteins shock play important roles in aquatic organisms including Artemia and other aquatic crustaceans (Chaurasia et al., 2015), potential applications for Hsps in the production commercial ofcrustaceans and other aquatic organisms are indicated (Sung et al., 2011), In the present study the effect of feeding

larvae and adult individuals of Artemia with identified stressed new Saccharomyces cerevisiae strain YG3-1 yeasts containing induced heat shock proteins 82 (Hsps82) as a novel way for enhancing the survival of Artemia in stress conditions was evaluated in larvae and adult individuals of Artemia urmiana and Artemia franciscana as test organisms. However, effects of live cerevisiae veasts (S.strains) supplementation on the performance of aquatic organisms have been studied previously (Perrone et al., 2013), but the effect of new identified S. cerevisiae strain YG3-1 yeasts containing induced Hsps82 on the survival of Artemia and other aquatic organisms has not been studied until now. Hsp82 is a member of Hsp90 family in yeast. Members of the Hsp90 family stabilize misfolded proteins and interact with regulatory signaling proteins in yeasts (Seppä, 2005) and play important roles in multiple cellular stress responses of aquatic organisms (Wang et al., 2016). Based on the findings mentioned previously, performed present study was investigate the effect of the function of Hsps82 (belonging to the S. cerevisiae strain YG3-1) on the survival of Artemia in stress conditions.

Materials and methods

Preparation of S. cerevisiae YG3-1 yeasts

All of the yeasts used in the present study were isolated from the intestine of endemic farmed rainbow trout (*Oncorhynchus mykiss*) in the West Azerbaijan province of Iran according

to the procedure as described previously (Andlid *et al.*, 1995), and then identified by molecular methods as a new strain of *S. cerevisiae* yeasts.

Induction of Hsp82 proteins in yeasts For this purpose, yeasts were cultured and grown using the yeast extractpeptone-glycerol (YPG) medium. In the stationary growth phase (after 3 days) were veast cells harvested centrifugation (5000 rpm for 10 m (minutes)) (Aoki et al., 2002). Then, harvested yeasts were divided into three groups and stored at -20 °C. After that, production of Hsps82 was stimulated in the two groups of stored yeasts. In this way, heat shocks (30, 35 and 40 °C) were applied on yeasts in one group of them. At the same time, yeasts in the other group were exposed to high salinity (60, 120, 180 and 240 g L⁻¹) as stress. Both stresses were performed for 4 h (hours) (Sathiyaa et al., 2001). Reverse transcription PCR (RT- PCR) was used to study the expression of hsps genes in the yeasts. Total RNA was isolated from each yeast sample single-strand cDNA and (complementary DNA) was synthesized from mRNA (messenger RNA that extracted from total RNA) using by 2steps RT-PCR-Kit. Before synthesis, RNA concentration and quality had been verified using Biophotometer (Eppendorf Biophotometer plus, Germany). Then, the cDNA fragments were amplified (using Cinnagen Co PCR-Kit) using specific hsp primers combination as a forward primer (Hsp82_{forward} 5'-AGT-TGC-CGA-CAG-AGT-TCA-GGT-TA-3') and a reverse

primer (Hsp82_{reverse} 5'-AGA-ACC-ACC-AGC-GTT-GGA-TT-3'). primers previously had been designed using theOligo software and produced by Cinnagene Co. PCR thermal cycling parameters for these specific primers were somewhat modified as: 35 cycles of 95 °C for 30 s (seconds), 45 °C for 40 s, 72 °C for 40 s with final extension of 72 °C for 5 min for amplification of hsp82. This program was applied with the Master cycler gradient Eppendorf thermal cycler. After amplification, all products were run on the 1.6% agarose electrophoresis, stained ethidium bromide and visualized in a UV- transilluminator contained CCD camera (Sankian et al., 2011).

Culture and feeding Artemia with yeast Hsps

Cyst samples (cysts of Artemia urmiana and Artemia franciscana) were obtained from the cyst bank of Urmia Lake Research Institute at Urmia University, Urmia, Iran. For optimal hatching, 1.5 g cysts of each population was incubated in artificial 0.45 µm filtered medium at a salinity of 35 g L⁻¹. After hatching, 500 individuals of instar-I nauplii were transferred directly into cylindroconical vials at an initial density of 2 nauplii ml⁻¹ of 80 g L⁻¹ culture medium (Sankian et al., 2011). Finally, according to the standard protocol culture was performed in two treatments and four replicates for each treatment. Air during culturing was passed through a 0.22-µm filter (Van Stappen et al., 1996). During the culture, both species of Artemia (A. urmiana and A. franciscana) were fed with two forms of yeast *S. cerevisiae YG3-1*: 1) Yeasts without any treatment (as Control treatment), 2) Yeasts with induced Hsp82 (combination of yeasts that were exposed to thermal and osmotic stresses as Hsp treatment), plus live *Dunaliella tertiolecta* algae. Feeding was performed in accordance with a feeding table which was adopted from a previously established procedure (Coutteau *et al.*, 1990).

Stress of Artemia; Determination of induced tolerance to stress in Artemia and statistical analysis

After the end of feeding, adult individuals of both species were exposed to high salinity (230 g L⁻¹ and 280 g L⁻¹) and high temperature (35 °C and 37 °C) as permitted stress for 48 h (hours). They were fed with live D.tertiolecta algae during the stress (Sung et al., 2008; Sankian et al., 2011). Stresses were applied based on guidelines (standard protocols adopted from previous literature) and in accordance with animals experimentations ethics and were approved by the Animal Experimentation Ethics Committee of the Urmia University, Urmia, Iran. To evaluate the effects of feeding with induced Hsps82 of S. cerevisiae strain YG3-1 yeasts on the survival and stress resistance of Artemia, numbers of swimming larvae were determined and the survival percentage was calculated. Mortality and survival were compared by means of one-way ANOVA. The results of survival were expressed as percentage. One-way ANOVA and Duncan's test of SPSS16 software were

used to identify differences among means, and significances were accepted at *p*<0.05 (Baxevanis *et al.*, 2004).

Protein extraction and sample preparation for SDS-PAGE

The whole body of adult Artemia which survived individuals from stresses was pulverized in 1.5 ml micro tube by appropriate tips. Then, 100 mg of the resulting powder, consisting of 10-15 adult Artemia individuals of both species, was homogenized in 500 µl of protein extraction buffer, containing buffer K (5 mM MgCl2, 5 mM NaH2PO4, 40 mM Hepes, 70 mM Potassium gluconate, 150 mM Sorbitol, pH 6.5) and protease inhibitor cocktail (InvitogeneTM Mini from Roche **Diagnostics** GmbH). Protein concentration was determined by the Bio photometer (Eppendorf Biophotometer plus, Germany). Samples were heated at 95 °C for 5 min and subsequently cooled. After that, insoluble fragments were removed by low speed centrifugation (1600 rpm for 5 min) and then supernatants were electrophoresed (Clegg et al., 2000; Sankian *et al.*, 2011).

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Flatbed SDS-PAGE was performed with a vertical system (BioRad System, USA). 10 % total acrylamide gel with $100\times70\times0.5$ mm dimensions was prepared. 30 μ l of samples were loaded

on each gel track (Clegg et al., 2000; Sankian et al., 2011). The running buffer was prepared according to the described procedure previously (Diezel et al., 1972). The buffer system in the strips formed a discontinuous buffer system together with the gel buffer. High molecular weight (250 KD) Ladder (Marker) was used for the detection of protein bonds on the gel. Electrophoresis was performed at a constant current of 50 mA (milliamper) in BioRad electrophoretic apparatus with power supply, set at 150V for 1 h. The gel was stained with Coomassie blue G250 (Clegg et al., 2000; Sankian et al., 2011).

Results

RT-PCR results

Investigation of gene expression in stressed yeasts has shown that under different temperature and salinity, the Hsps gene expression of these yeasts has changed. So, the stress enhances the mRNA transcripts of Hsps in stressed yeast cells. RT-PCR gel electrophoresis of hsps genes expression indicated that 4h exposure to all of the stresses resulted in expression of hsps82 genes in S. cerevisiae YG3-1. Intensity of cDNA bands has shown that the temperature 35 °C and salinity 60 g L⁻¹ are the best conditions for expression of Hsps genes in S. cerevisiae YG3-1 (Fig. 1).



Figure 1: Semi quantitative RT-PCR gel electrophoresis of HSP 82 expression in various samples of Saccharomyces cerevisiae YG3-1. M: Marker (1 Kb ladder), lane 1: expressed HSP at 30 °C, lane 2: 35 °C, lane 3: 40 °C, lane 4: expressed HSP at salinity 60 g L⁻¹, lane 5: 120 g L⁻¹, lane 6: 180 g L⁻¹, lane 7: 240 g L⁻¹, lane 8: negative sample, lane 9: control. Semi quantitative RT-PCR revealed that the stress enhances the mRNA transcript of Hsp in stressed yeast cells. Intensity of bands shows the temperature 35 °C and salinity 60 g L⁻¹ are the best conditions for expression of Hsps genes.

Enhanced survival of Artemia in stress conditions

Survival of *Artemia* individuals that were fed with Hsps in stress conditions significantly was improved and enhanced as compared to control *Artemia* individuals fed with non-

stressed yeasts (p<0.05). So among the stressed *Artemia* adult individuals, the minimum percentage of survival in individuals of *A.urmiana* wasn't less than 60 % and Hsp-fed individuals in *A.franciscana* samples exhibited the highest survival (Table 1).

Table 1: Survival of Artemia urmiana and A. franciscana.

Treatment	Day 7	Day 11	Day 15
Control/ A. urmiana	89.50±17.90 ^a	80.35±16.05 ^a	77.75±15.55 ^a
HSP/A. urmiana	89.99±17.99 ^a	83.35 ± 16.69^{a}	80.95±17.55 ^a
Control/A. franciscana	89.75±17.55 ^a	82.50 ± 16.40^{a}	78.45 ± 15.69^{b}
HSP/A. franciscana	91.75±14.94 ^b	89.75±17.55 ^b	81.80±16.16 ^a

Same characters in each column indicate insignificant differentiation. Hsp-fed individuals in *A. franciscana* samples exhibited the highest survival. The results are presented as the average of three determinations with standard deviations (mean±SD).

Synthesis of Hsps in Artemia

Staining of SDS polyacrylamide gels (SDS-PAGE of the survived adult *Artemia* in high salinity and high temperature) clearly demonstrated increased amounts of almost 80-kDa polypeptides in *Artemia* samples fed with yeast Hsps. While, *Artemia* individuals that fed with control yeast cells (non- stressed yeast cells with no Hsp) do not produce Hsp (Fig. 2).

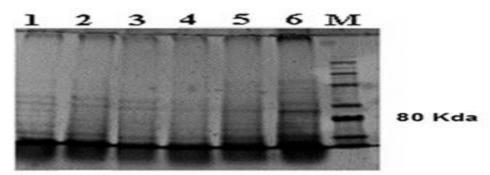


Figure 2: SDS-PAGE of total protein of the survived adult *Artemia* in different feeding treatments: 80 kDa protein bands were resolved in 10% SDS polyacrylamide gel. Lane 1: *A. franciscana* fed with Control treatment, lane 2: *A. franciscana* fed with HSP treatment, lane 3: *A. franciscana* fed with HSP treatment, lane 4: *A. urmiana* fed with Control treatment, lane 5: *A.urmiana* fed with HSP treatment, lane 6: *A. urmiana* fed with HSP treatment, M: Marker (250 KD ladder). As revealed by SDS-PAGE, administration of yeast Hsps enhances Hsp production in whole *Artemia* fed with Hsp. *Artemia* individuals that fed with control yeast cells (non- stressed yeast cells with no Hsp) do not produce 80-kDa polypeptides.

Discussion

The results of this study showed that feeding Artemia with induced Hsps of S. cerevisiae resulted in the induction of Hsps production in Artemia and confirmed that this feeding resulted in resistance of Artemia against stress. following Maybe the dietary administration with stimulated Hsps82 of S. cerevisiae strain YG3-1 yeasts, chaperone activities of Hsps82 in Artemia induced and were these activities have resulted in stress tolerance in Artemia. Before this, ingestion of E. coli over-producing Artemia Hsp70 shelters brine shrimp against V. campbellii, possibly by triggering the innate immune response anti-inflammatory to produce substances and suppress infection. DnaK and Artemia Hsp70 exhibit 59.6% similarity in the peptide-binding domain and the protective capacity of these proteins, termed the innate immunity-activation portion, may reside within this molecular domain, a conclusion similar to that made for Hsp70 from dendritic cells (Sung et al., 2009b; Sung and Macrae, 2011). In the present study, maybe there was a similarity in the domain of yeast Hsp82 and Artemia Hsps (that following the feeding with Hsp-enriched yeasts made from hemocytes of Artemia). Possibly, this similarity, has resulted in the induced-production of Hsps in Artemia. In addition, Hsps appear to stimulate the innate immune response of aquatic organisms and reinforce the function of the immune system (Sung and Macrae, 2011). Reinforcement of immune system function helps resistance against stressors and therefore enhances the survival in stress conditions (Aleng et al., 2015). In the present study, yeast Hsps82 may reinforce the immune system function of Artemia individuals and so enhance their survival against stress conditions (hyperthermal and hyperosmotic stresses). Families of heat shock proteins, otherwise known as stress proteins or molecular chaperones,

consist of conserved molecules found in all organisms. The expression of genes encoding Hsps is either constitutive or induced by stress and their products are essential for cell survival. Under normal conditions Hsps mediate nascent and protein folding assembly, translocate proteins through membranes into organelles such as mitochondria, and assist in the degradation of structurally aberrant proteins. Hsps, often when functioning cooperatively with one another, prevent irreversible denaturation of proteins exposed to physiological stressors such as heat, toxins and disease, thereby facilitating protein refolding and protecting cells from damage (Sung and Macrae, 2011). Heat shock proteins were reported to inducte and enhance the resistance of aquatic invertebrates including crustaceans such as Artemia shrimp against environmental stress. Also, previous studies showed that Hsps can be induced by external stresses. For example, expression of 90 KDa heat shock proteins in the brine Artemia Leach shrimp 1819. response to high salinity stress protected Artemia against the high salinity stress (Sankian et al., 2011). In addition, exposure of gnotobiotic A. franciscana larvae to abiotic stress (hypothermic and hyperthermic shocks) promoted heat shock protein synthesis and enhanced resistance to pathogenic V. campbellii and showed a causal link between Hsp70 accumulation induced by abiotic stress and enhanced resistance to infection by V. campbellii, perhaps via stimulation of the Artemia immune system (Sung et

al., 2008). In many studies heat shock proteins have been used to enhance the stress tolerance in aquatic crustaceans such as Artemia and shrimp. Heat shock proteins can not only be produced by stress in all living animals, but can also be absorbed externally. Feeding of bacterially encapsulated heat shock proteins to invertebrates is a novel way limit Vibrio infection. As example, ingestion of Ε. coli overproducing prokaryotic Hsps significantly improves survival gnotobiotically cultured Artemia larvae upon challenge with pathogenic V. campbellii (Sung et al., 2009b). Moreover, feeding with non-pathogenic strains of E.coli including YS1, YS2 (containing 70-kDa bacterial Hsp., DnaK) and Anative (containing Artemia Hsp70 cDNA) containing induced Hsp70 proteins conferred protection to A. franciscana individuals against V. campbellii infection and enhanced their length (Baruah et al., 2010). In a similar study, feeding with bacterial heat shock protected A. protein franciscana (Kellogg) larvae from V. campbellii infection (Sung et al., 2009a). The described experiments showed HSPs can elevate survival performance of aquatic crustaceans in stressor conditions. Also, considering these results, feeding with yeast heat shock proteins can be considered as a novel way to make Artemia and other aquatic crustaceans resistant against stress conditions. As mentioned previously, effect of feeding with S. cerevisiae strain YG3-1 yeasts containing induced heat shock proteins 82 on the survival of Artemia in the present study was evaluated and resulted in resistance of Artemia urmiana and Artemia franciscana (as a model organisms) against abiotic stress. The results of this study suggest that described yeasts can be used to protect the other species of Artemia from various abiotic stresses during culture. Previously, effects of βglucan polysaccharides of mercaptoethanol (2ME)treated YG3-1 S.cerevisiae strain the growth of Artemia were investigated (Shekarchi et al., 2016), but the effect of Hsps82 of S.cerevisiae strain YG3-1 on the survival of Artemia in stress conditions has not been studied until now. So, considering the adverse side effects of antibiotics on the public health of aquatic organisms in the long term (Nikkhoo et al., 2010), this administration can be used as a novel safe way to enhance the performance (improving growth and survival) of farmed Artemia during the culture.

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References

Aleng, N.A., Sung, Y.Y., H.MacRae, T. and Abd Wahid, M.E., 2015.

Non-lethal heat shock of the Asian green mussel, *Perna Viridis*,

promotes Hsp70 synthesis, induces thermotolerance and protects against *Vibrio* Infection. *Plos One*, 10(8), 1-16.

Andlid, T., R.V. Juarez, and Gustafsson, L., 1995. Yeast intestine colonization the of Rainbow trout (Salmo gairdneri) and Turbot (Scophtalmus maximus). Microbial Ecology, 30, 321-334.

Aoki, H., Miyamoto N., Furuya Y., Mankura M., Endo, Y. and Fujimoto, K., 2002. Incorporation and Accumulation of Docosahexaenoic Acid from the Medium by *Pichiamethanolica* HA-32. *Bioscience Biotechnology Biochemistry*, 66, 2632-2638.

Arkush, K.D., Cherr, G.N. and Clegg J.S., 2008. Induced thermotolerance and tissue Hsc70 in juvenile Coho salmon, *Oncorhynchus kisutch. Acta Zoologica*, 89, 331–338.

Baruah, K., Ranjan, J., Sorgeloos, P. and Bossier, P., 2010. Efficacy of heterologous and homologous heat shock protein 70s as protective Agents to *Artemia franciscana* challenged with *Vibrio campbellii*. *Fish and Shellfish Immunology*, 29, 733-739.

Baxevanis, A.D., El-Bermawi, N., Abatzopoulos, T.J. and Sorgeloos, P., 2004. International Study on Artemia. LXVIII. Salinity effects on maturation, reproductive and life span characteristics of four Egyptian Artemia populations. Hydrobiologia, 513, 87-100.

Chaurasia, M.K., Nizam, F., Ravichandran, G., Arasu, M.V., Al-Dhabi, N.A., Arshad, A.,

- Elumala, P. and Arockiaraj, J., 2015. Molecular importance of prawn large heat shock proteins 60, 70 and 90. Fish and Shellfish Immunology, 48, 228-238.
- Clark, S.M., Fraser K.P.P. and Peck, S.L., 2008. Lack of an HSP70 heat shock response in two Antarctic marine invertebrates. *Polar Biology*, 31(9), 1059-1065.
- Clegg, J.S., Jackson, S.A., Hoa, N.V. and Sorgeloos, P., 2000. Thermal resistance, developmental rate and heat shock proteins in *Artemia franciscana*, from San Francisco Bay and Southern Vietnam. *Journal of Experimental Marine Biology and Ecology*, 252, 85–96.
- Coutteau, P., Lavens, P. and Sorgeloos, P., 1990. Baker's yeast as a potential substitute for live algaein aquaculture diets: *Artemia* as a case study. *Journal of the World Aquaculture Society*, 21, 1-9.
- Diezel, W., Kopperschläger, G. and Hofmann, E., 1972. An improved procedure for protein staining in polyacrylamide gels with a new type of Coomassie Brilliant Blue. *Analytical Biochemistry*, 48, 617–620.
- García, C.R., Hasanuzzaman, A.F.M., Sorgeloos, P. and Bossier, P., 2008. Cell wall deficient Saccharomyces cerevisiae strains as microbial diet for Artemia larvae: Protective effects against vibriosis and participation of phenoloxidase. Journal of Experimental Marine Biology and Ecology, 360, 1–8.
- Gavanda, R.M., McClintocka, B.J., Amslera, D.C., Petersb, W.R. and

- Angusa, A.R., 2007. Effects of sonication and advanced chemical oxidants on the unicellular green alga *Dunaliella tertiolecta* and cysts, larvae and adults of the brine shrimp *Artemia salina*: A prospective treatment to eradicate invasive organisms from ballast water. *Marine Pollution Bulletin*, 54, 1777–1788.
- Givskov Sørensen, J., 2010.

 Application of heat shock protein expression for detecting natural adaptation and exposure to stress in natural populations. *Current Zoology*, 56(6), 703–713.
- Gunderson, R.A., Armstrong, J.E. and Stillman, H.J., 2016. Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. Annual Review of Marine Science, 8, 12.1-12.2.
- Li, N., Zhang, L., M. Zhang, K.Q., Deng, J.S.H., Prandl, R. and Schöffl, F., 2006. Effects of heat stress on yeast heat shock factor-promoter binding *in vivo*. *Acta Biochimica et Biophysica Sinica*, 38(5), 356–362.
- Nikkhoo, M., Yousefian, M., Safari, R. and Nikkhoo, M., 2010. The influence probiotic of aqualase on the survival, growth, intestinal microflora and challenge infection in wild carp (*Cyprinius Carpio L.*). Research Journal of Fisheries and Hydrobiology, 5(2), 168-172.
- Perrone, G.M., Perez, A., Civiglia, J. and Chiape Barbara, A., 2013. Effects of live yeast (*Saccharomyces*

- cerevisiae Strain 1026) supplementation on the closure of articular growth plates in quarter horse foals. *Journal of Equine Veterinary Science*, 33, 261-265.
- Qin, C., Shao, T. and Duan, H., 2016. The cloning of a heat shock protein 90 gene and expression analysis in *Botia reevesae* after ammonia-N exposure and *Aeromonas hydrophila* challenge. *Aquaculture Reports*, 3, 159-165.
- Sankian, Z., Heydari, R. and Manaffar, R., 2011. Expression of 90 KDa heat shock proteins in the brine shrimp Artemia Leach, 1819 (Crustacean: Anostraca) in response to high salinity stress. International Journal of Artemia Biology, 1, 3-12.
- Sathiyaa, R., Campbell, T. and Vijayan, M., 2001. Cortisol modulates HSP90 mRNA expression in primary cultures of trout hepatocytes. *Comparative Biochemistry and Physiology Part B*, 129, 679-685.
- **Seppä, L., 2005.** Regulation of heat shock response in yeast and mammalian cells. Dissertations bioscientiarum molecularium Universitatis Helsingiensis in Viikki. 17/2005, 59 P.
- Shekarchi, B., Nekuiefard, A. and Manaffar, R., 2016. The effects of dietary administration with chemical treated *Saccharomyces cerevisiae* strain *YG3-1* on the growth of aquatic invertebrates in *Artemia*. *Journal of Coastal Life Medicine*, 4(2), 128-131.

- Shi, H.N., Liu, Z., Zhang, J.P., Kang, Y.J., Wang, J.F., Huang, J.Q. and Wang, W.M., 2015. Effect of heat stress on heat-shock protein (Hsp60) mRNA expression in rainbow trout *Oncorhynchus mykiss. Genetics and Molecular Research*, 14(2), 5280-5286.
- Sung, Y.Y., Pineda, C., MacRae, H.T., Patrick Sorgeloos, P. and Bossier, P., 2008. Exposure of gnotobiotic Artemia franciscana larvae to abiotic stress promotes heat shock protein 70 synthesis and enhances resistance to pathogenic Vibrio campbellii. Cell Stress and Chaperones, 13, 59–66.
- Sung, Y.Y., Ashame, M.F., Chen, S., H.MacRae, T., Sorgeloos, P. and Bossier, P., 2009a. Feeding *Artemia franciscana* (Kellogg) larvae with bacterial heat shock protein, protects from *Vibrio campbellii* infection. *Journal of Fish Diseases*, 32(8), 675-685.
- Sung, Y.Y., Dehaene, T., Defoirdt, T., Boon, N., MacRae, H.T., Sorgeloos, P. and Bossier, P., 2009b. Ingestion of bacteria overproducing DnaK attenuates Vibrio infection of Artemia franciscana larvae. Cell Stress and Chaperones, 14, 603-609.
- Sung, Y.Y. and MacRae, H.T., 2011.

 Heat shock proteins and disease control in aquatic organisms.

 Journal of Aquaculture and Research Development, 3, 1-10.
- Sung, Y.Y., H. MacRae, T., Sorgeloos, P. and Bossier, P., 2011. Stress response for disease control in

- aquaculture. *Reviews in Aquaculture*, 3, 120-137.
- Sung, Y.Y., Roberts, R.J. and Bossier, P., 2012. Enhancement of Hsp70 synthesis protects common carp, *Cyprinus carpio* L., against lethal ammonia toxicity. *Journal of Fish Diseases*, 35, 563–568.
- **Tkáčová, J. and Angelovičová, M., 2012.** Heat shock proteins (HSPs): A review. *Animal Science and Biotechnologies*, 45(1), 349-353.
- Van Stappen, G., Lavens, P. and Sorgeloos, P., 1996. Artemia In: Manual on the production and use of live food for aquaculture. Ghent (BEL): Aquaculture and Artemia Reference Center, University of Ghent (BEL). FAO Publication. No. 361.
- Wang, P.F., Zeng, Sh., Zhou, L. and Li, G.F., 2016. Two HSP90 genes in mandarin fish *Siniperca chuatsi*: identification, characterization and their specific expression profiles during embryogenesis and under stresses. *Fish Physiology and Biochemistry*, 42, 1-14.
- Xu, D., Sun, L., Liu, S., Zhang, L. and Yang, H., 2016. Understanding the Heat Shock Response in the Sea Cucumber *Apostichopus japonicus*, Using iTRAQ-Based Proteomics, *International Journal of Molecular Sciences*, 150(17), 1-13.