

Sponges Lubomirskiidae as bioindicators of the state of Lake Baikal

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ABSTRACT. Sponges are ideal bioindicators of the environmental state due to their simple body structure, filter-feeding lifestyle and widespread abundance. For marine sponges, stress response studies are conducted in the field of microbial composition, gene expression and transcriptome techniques. For freshwater sponges expression of the stress protein HSP70 was studied. In the Baikal sponges, the content of HSP70 is an indicator of the stress response to temperature increase. Transcriptomic studies of the endemic Baikal sponges are in progress.

Keywords: Porifera, bioindicators, Lake Baikal, Lubomirskiidae, HSP70, hyperthermia

1. Introduction

Sponges (Porifera) are the earliest multicellular animals and important component of marine and freshwater ecosystems (Diaz and Rützler, 2001; Bell, 2008; Van Soest et al., 2012; Webster et al., 2013). Sponges show remarkable ecological adaptability and they were able to inhabit all aquatic ecosystems of the Earth.

Sponges are ideal bioindicators of the environmental state, due to their simple body structure, species richness, filter-feeding life style and widespread abundance. Sponges pump large quantities of water and have ability to concentrate a wide range of chemicals from both the suspended and dissolved phases of the water (Orani et al., 2018). Additionally, sponges are highly tolerant of some pollutants and have detoxification systems, making them suitable model organisms for monitoring studies (Orani et al., 2018).

For all monitoring programs, it is important to identify monitoring parameters and to determine the most appropriate indicators. In particular, for sponge monitoring a focus on specific sponge populations with estimates of abundance and taxonomic composition at regular intervals should be a important part of the program (Bell et al., 2017). Sponge tissue can be sampled periodically for microbial community composition, metabolite production and stress protein expression, which will provide a large amount of data. The studies of the influence of changes in environmental conditions

on these parameters are limited, although sponges have shown to be very sensitive to changes in surrounding conditions (López-Legentil et al., 2008; Pantile and Webster, 2011).

For marine sponges, stress response studies were conducted in the field of microbial composition, gene expression and transcriptome techniques. The understanding of molecular mechanisms of the sponge stress response is poor, since most studies focus on the effect of stress on the sponge-associated microbial community (Selvin et al., 2009; 2010; Kiran et al., 2018). However, the microbial composition has a large intraspecific and interspecific variability, as well as varies by depth and season (Selvin et al., 2009).

Changes in the gene expression patterns of stress proteins can serve as a biomarker to assess levels of acute stress and determine the environmental load on a sponge (Bell et al., 2017). Heat shock proteins (Hsps) play an important role in maintaining protein homeostasis during adaptation of organisms to variable environmental conditions (Parsell and Lindquist, 1993; Feder and Hofmann, 1999; Tomanek, 2010). Previous studies demonstrated enhanced transcription of HSP70 in marine sponges undergoing temperature stress, osmotic shock, pH stress, heavy metals and phenols stress (Schröder et al., 2006; López-Legentil et al., 2008; Webster et al., 2013).

The studies on the stress response of freshwater sponges are rare. HSP70 expression was first shown in the freshwater sponge *Ephydatia fluviatilis* (Müller

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et al., 1995). In thermally stressed *Spongilla lacustris* HSP70 level patterns resemble those of gene expression patterns and exhibit an even greater intensity and sensitivity (Schill et al., 2006). In gemmules (resting bodies that contain totipotent cells) of the freshwater sponge *S. lacustris*, increased levels of cellular HSP70 and hsp70 mRNA likely allow the gemmules to stabilize their proteins and membranes when the water temperature changes (Schill et al., 2006). Increased HSP70 level was detected in the Lake Baikal sponges *Lubomirskia abietina* and *Baikalospongia intermedia* after exposure at the elevated temperature of 20°C, waste water from the Baikal Pulp and Paper Mill, lead and zinc, while copper had no effect (Efremova et al., 2002; Schröder et al., 2006).

Lake Baikal is the oldest and deepest lake in the world. Its age is estimated at 35 million years, and its maximum depth has been recorded at 1,647 meters. Endemic species in the lake evolved into species flocks and form special mechanisms for adaptation. The Lake Baikal endemic sponge of the family Lubomirskiidae constitutes the bulk of benthic biomass and includes thirteen described species and two subspecies (Efremova, 2001; 2004; Itskovich et al., 2017). *Lubomirskia baicalensis* inhabits depths of 3-120 meters and dominates the littoral zone of the lake (Masuda, 2009). We analyzed the effect of increasing temperatures on the dynamics of the HSP70 level in Baikal endemic sponge *Lubomirskia baicalensis* to evaluate this marker as a possible bioindicator.

2. Materials and methods

Samples of *L. baicalensis* were collected by SCUBA diving in August 2013 in the Southern basin of Lake Baikal near the Bol'shoye Koty settlement at a depth of 10 m. Immediately after transfer to the laboratory, sponge specimens (n = 3) were placed into an aquarium with Baikal water and were kept at 4°C with 12h/12h light-dark cycles and aeration for 14 days to achieve acclimatization (Fig. 1). One part (5 cm lengths) of each sponge was cultivated in a 6 L aquarium at 9°C and 13°C with daily water exchange. The controls were continuously kept at 4 °C for one month. Temperatures of 9°C and 13°C were chosen according to data on the variation of the fatty acid composition of total lipids in *L. baicalensis* during aquarium cultivation at these temperatures (Glyzina and Glyzin, 2014). Incubation times were 2 h, 15 h and 7 days. Subsequently, small parts (in triplicate samples) of each sponge were frozen in liquid nitrogen.

Total protein was extracted from three replicates of each sample frozen in liquid nitrogen immediately after the temperature treatment. Protein from the *L. baicalensis* sponge was extracted as described previously (Voinikov et al., 1986). Protein concentration in the samples was determined with Quant-iT™ Protein Assay Kit (Thermo Fisher Scientific). 30 µg of protein from each sample were separated by electrophoresis in 12% SDS-PAGE (Laemmli, 1970) then the protein was transferred onto a nitrocellulose membrane in a



Fig. 1. Endemic Baikal sponge *Lubomirskia baicalensis* (Pallas, 1773)

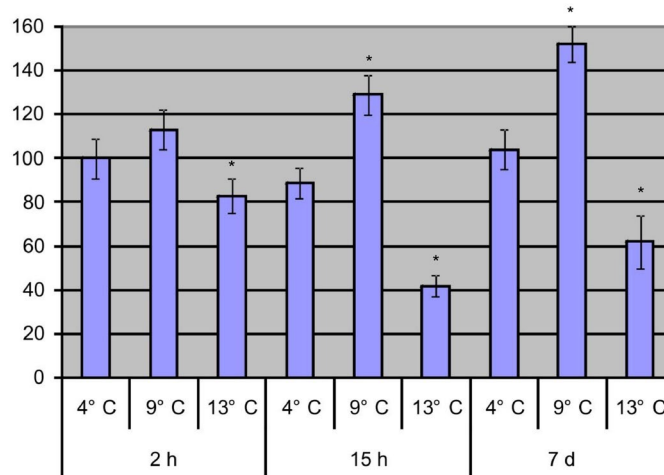


Fig. 2. The relative HSP70 levels in *L. baicalensis* exposed to thermal stress at 9 °C and 13 °C for 2 hours, 15 hours and 7 days. The HSP70 level of sponge kept at 4 °C for 2 hours was estimated as 100%. Means ± SD. Asterisks indicate significant difference ($p < 0.05$) from the control

mini-Protean III (Bio-Rad, USA) system following the manufacturers protocol.

HSP70 was detected with antibodies, as described previously (Timmons and Dunbar, 1990). Primary antibodies against HSP70 were used (Cat. No H5147 «Sigma», USA). The antibodies were visualized with secondary antibodies conjugated with alkaline phosphatase («Sigma», USA). The intensity of spot coloring was determined with Gel Analysis software and expressed as percentage of intensity to the spot coloring of the protein in the control (= 100%, left bar in figures) as previously described (Itskovich et al., 2018).

Statistical analysis included mean ± SD, normality test (Shapiro-Wilk), and one-way analysis of variance (ANOVA) with SigmaPlot (V12.0, SysStat Software Inc., Ca, USA). Statistical significance was tested by the Fisher LSD method.

3. Results and discussion

To study the effect of elevated temperatures on the dynamics of HSP70 accumulation in the Baikal endemic sponge *L. baicalensis* we used two elevated temperature. Constitutive synthesis of HSP70 was detected in samples immediately after sampling. After acclimatization and further keeping in the aquarium during 1 month at 4 °C we did not detect increased Hsp70 levels, which indicate appropriate cultivation conditions.

We have determined that after the temperature rise from 4°C to 9°C the HSP70 level increased in 2 hours to 110% compared to the control, and in 15 hours it further increased to 130% (Fig. 2). However, when the temperature rose to 13°C, the HSP70 level decreased in 2 hours to 80% and in 15 hours – to 40% in comparison with the control. After 7 days of incubation at 9°C the HSP70 level increased to 150% compared to the control (Fig. 2). Thus, the rising temperature to 9°C increased the HSP70 level in *L. baicalensis*, but the temperature rise to 13°C reduced the HSP70, indicating the inhibition of metabolism.

Our experimental data have shown that the Baikal sponges respond to a rise in temperature by changing the content of HSP70. The detected temperature optimum of *L. baicalensis* associated with the environmental conditions of its natural habitat. *L. baicalensis* is a littoral species inhabiting the depths of 3-120 m (Efremova, 2001; Masuda, 2009). The water temperature in the lake at a depth below 250 m is a constant 3.3 - 4.3°C, but during warming up periods in summer it can reach 12-13 °C in some places at 10 m depth (Shimaraev et al., 1994; Timoshkin et al., 2009). In bays and the Maloye More Strait (Central Baikal) temperature fluctuations are most significant, from 0.1 to 25°C (Kozhova and Izmet'eva, 1998).

Along with the experimental data, the development of bioindicators and assessment of the state of the lake ecosystem requires research in natural conditions. The study of the stress response of the Baikal sponges is relevant in connection with a mass sponge bleaching event which has been detected recently (Kaluzhnaya and Itskovich, 2015; Timoshkin et al., 2016; Itskovich et al., 2018; Khanaev et al., 2018; Kulakova et al., 2018). In situ sponge surveys should be performed to estimate HSP70 level and gene expression and these data can be used to assess the health status of sponges in Baikal. In marine sponge *Xestospongia muta*, two types of bleaching have been described: cyclic bleaching, from which sponges can recover, and fatal bleaching, which usually results in sponge death (López-Legentil et al., 2008). Unlike cyclically bleached tissues, fatally bleached samples had higher hsp70 gene expression. At the same time both cyclic and fatally bleached tissues had lower chlorophyll a concentrations than unbleached tissue. Therefore hsp70 expression was a better indicator between fatal and cyclical bleaching, compared to chlorophyll a content (López-Legentil et al., 2008). Our preliminary study revealed the decreased HSP70 level of in *L. baicalensis* both in the case of bleaching after exposure to 13 °C,

and in diseased individuals from Lake Baikal (Itskovich et al., 2018). At present, little is known about the HSP70 production in response to chronic stress, since most studies deal with acute stress of Porifera (Bell et al., 2017). Mass sponge disease in Lake Baikal indicates that sponges are subject to chronic stress in the lake. Longer observations of the HSP content and gene expression in diseased and healthy sponges from Lake Baikal can provide information about their ability to recover.

The complete information about changes in gene expression can be obtained by transcriptome-wide survey. Study of gene expression dynamics in the shallow-water sponge *Haliclona tubifera* by high-throughput transcriptome sequencing revealed activation of various processes that interact to maintain cellular homeostasis during stress response to elevated temperature (Guzman and Conaco, 2016). Heat shock proteins, antioxidants, and genes involved in signal transduction and innate immunity pathways were upregulated after short-term thermal stress whereas prolonged exposure resulted in higher expression of genes involved in cellular damage repair, apoptosis, signaling and transcription (Guzman and Conaco, 2016). While in *L. baicalensis* HSP70 level was decreased at sublethal temperature, in *H. tubifera* the relative expression of Hsp70 was strongly upregulated during exposure to sublethal temperature (Guzman and Conaco, 2016). This may indicate that *L. baicalensis* has greater sensitivity to thermal stress than *H. tubifera* due to living in more stable conditions. These results may also indicate differences in gene expression and protein content of HSP70 due to posttranscriptional regulation. Combining transcriptome data with those on protein content would provide important information about the stress response of sponges. Transcriptomic studies of the endemic Baikal sponges are in progress.

4. Conclusion

HSP70 is a molecular indicator which can provide early identification of environmental stress on aquatic communities. Our data have shown that the changes in HSP70 content accurately reflect the stress response to elevated temperature in *L. baicalensis* and, therefore, is indicative and can serve as an early marker of environmental stress. The in situ studying of chronic stress by evaluating gene expression and protein content would contribute to a better understanding of the potential mechanisms of adaptation, resilience and conservation of sponges.

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