Short communication

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Fatty acid composition as one of the markers of state and functioning of branching Baikal sponge

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ABSTRACT. The fatty acid composition of visually healt*h and sick Lubomirskia b*aicalensis sponges from Lake Baikal (years of 2016 and 2018) was determined. Sponges collected in 2018 had an abnormality of demospongic acid synthesis. Demospongic acids are of antibiotic activity and protect sponges. *In vitro*, there was an increase in the concentrations of the palmitic, oleic, linoleic and α-linolenic sponge acid under stress. These substances are responsible for cell membrane repairing and antibiotically active. During three weeks, there were also demospongic acid synthesis termination and appearance of disease precursors was noted.

Keywords: Lake Baikal, sponge*, Lubomirskia baicalensis*, FAME, GC-MS

Lubomirskia baicalensis is a freshwater endemic Lake Baikal sponge of the Lubomirskiidae family. It covers the stony bottom of the littoral benthic zone at depths from 3–4 up to 30–40 m. Due to of the great biomass of Lubomirskiidae in Lake Baikal and ability of sponge to pass large volumes of fresh water through its body, the filtration activity of sponges is of great importance in the processes of purification of a water reservoir (Kupchinsky et al., 2019). Sponge skeleton has unique physical-chemical properties and can be an adsorbent with high sorption capacity, as our experiments show. Analysis *in situ* of sorption properties of the Baikal *L. baicalensis* shows that the sponges can sorbs up to 0.1–0.5 mg of synthetic organic pollutants per 1 g dry weight of sponge dried at room temperature during 72 h ($n = 25$).

The appearance of abnormal pink sponges in Lake Baikal was recorded in 2011 (Bormotov, 2011). By 2014, diseases and mortality of different sponge species have increased rapidly and covered the littoral zone through the lake perimeter (Khanaev et al., 2018). Causes of disease, as well as mechanisms of functioning and adaptation of sponges and other species of low phylogenetic levels have poorly studied yet. Different causes of disease can be suggested such as anthropogenic effect and following microbial invasion. Biochemical analysis and experiments *in situ* and *in vitro* can clear a reason of this phenomenon. There are numerous studies on sponge fatty acids (Latyshev et al., 1992; Rod'kina, 2005; Glyzina et al., 2016*)*. To understand processes in organisms of visually health and sick sponges we analyzed fatty acid composition using high accuracy analytical techniques.

Sponge samples were taken in 2016 and 2018 in the southern basin of Lake Baikal. Some samples were placed in aquariums under conditions close to natural. Samples were prepared according to (Nikonova et al., 2020). Lipids were extracted with 1.2 mL of *Folch* mixture using an ultrasound bath for 5 min, and 0.35 mL of distilled water was added. Then the mixture was emulsified and centrifuged. Recovery was ~95%. Fatty acid (FA) methylation was carried out under soft conditions (55 °C, 1.5 hr, 2-% sulfuric acid solution in methanol). Esterified FAs were methylated separately in alkaline medium at 25 °C. Methyl esters of FAs (FAMEs) were analysed using 6890B GC System, 7000C GC/MS Triple Quad (Agilent, USA) and GC-2010 Plus coupled with FID (Shimadzu, Japan) with Optima-17MS GC-columns (30 m × 0.25 mm, Macherey-Nagel, Germany).

In 2016, FAME content in *L. Baicalensis* samples ranged from 8 to 35 mg/g of dry weight, and more than 60 FAs were found, with 34 FAs being identified reliably. They were saturated (SFA), branched, monounsaturated, polyunsaturated (PUFAs) and demospongic acids with 24–30 carbon atoms, that agrees with (Dembitsky et al., 1993). The SFA content was ~20% for visually healthy sponges and ~30% – for sick ones. The bacterial FAs content was ~4% for all samples, whereas the PUFA content, including the demospongic acid content, was 2–5 times higher for healthy sponges than for sick ones. In this case, the bacterial FAs content may show the similar state of bacterial symbionts of healthy and sick sponges, whereas the differences in the PUFA content, including demospongic FAs synthesized directly by sponge, might show differences in them. In 2018, the

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FAME content in *L. Baicalensis* samples was in ranged from 6 to 25 mg/g dry weight and only 15 FAs were found comparing to 2016. The SFA content was ~30%. Demospongic acids were not found. The free fatty acid (FFA) content was ~30–50 % that points to high stress.

The FFA content in sponges placed in aquariums at 0–3.6 ˚C increased under stress. After two weeks, FFA were not found. Every week we cut sponge branches and soon the visual state of sponges became worse and signs of disease appeared. The FFA content increased up to 80 times during a month. The highest increase was observed for palmitic, linoleic, oleic and α-linolenic FAs. Palmitic acid is one of the main components of cell membranes and its content growth can be explained by the repairшtп of damaged sponge cells. Oleic acid is an intermediate product for synthesis of demospongic FAs from palmitic acid (Latyshev et al., 1992; Dembitsky et al., 1993; Kornprobst and Barnathan, 2010). Two other acids (Dembitsky et al., 1993; Kornprobst and Barnathan, 2010) are of high antimicrobial activity. Also α-linolenic FA can repair destroyed cells. Demospongic FAs were not found in sponges placed in aquariums, as well as in natural ones (2018), though they are of high antibacterial and antifungal activity (Kornprobst and Barnathan, 2010). We can show *in vitro* and *in situ* an abnormal synthesis of demospongic acids that most likely causes intense microbial invasion. Stress factor for sponges *in situ* which destroy their immunity has not clearly determined yet.

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