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SHORT COMMUNICATIONS

Changes in the Lipid Composition of Freshwater Sponges upon Rise in Habitat Temperature

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The life of organisms is limited to a specific temperature range in which, theoretically, a 10°C rise in temperature leads to a two- to fourfold increase in the response time of metabolic processes.

As shown in numerous physical-chemical studies, the mechanisms of survival at abnormally high temperatures include changes in the lipid components of cell membranes, with the degree to which lipids are involved in the processes of adaptation depending on biological features of given species, including marine and freshwater sponges (*Temperature*..., 1994; Medeot et al., 2007; Velansky and Kostetsky, 2009; Gladyshev et al., 2011).

Because of increasing anthropogenic impact on natural ecosystems, adaptations developed by various organisms have recently received growing attention from researchers, but adaptive biochemical characteristics of organisms representing taxa of lower phylogenetic ranks are still poorly studied. Sponges as a symbiotic community of various microorganisms are a unique object for such studies.

During millions of years, Baikal sponges have adapted to living in a narrow temperature range from 0.5 to 11.5°C (at depths of 4 m and below). Every sponge and its endosymbionts (bacteria, microalgae, etc.) have a characteristic composition of lipids and particularly of fatty acids (FA), their major components and specific markers of vital activity. Thus, as the temperature rises, symbiotic bacteria show an increase in the percentage of saturated and branched-chain fatty acids (C17–C19), which improves cell membrane resistance. Bacteria also supply sponges with saturated and monoenoic acids with a chain length of up to C20. Suppression of symbiotic algae leads to the accumulation of C14 to C20 fatty acids, with C16:0 and C18:0 contents being doubled. Finally, unsaturated acids transform into saturated ones and then converted into aldehydes, vinyl ketones, dehydrogenases, saturated aldehydes, ketones, as well as saturated and unsaturated alcohols; the latter are responsible for a specific odor of decaying sponges. Lipids of sponges themselves are primarily distinguished by the presence of C24–C30 fatty acids. These unique demospongic acids are synthesized only in sponge cells and have one double bond in Δ5 position (Latyshev et al., 1992). Biosynthesis of these compounds is typical for the most evolutionally ancient animals.

In this paper, we describe changes in the lipid composition of the Baikal freshwater sponge *Baicalospongia bacillifera* upon a rise of temperature in its natural habitat by 6°C (supposedly, close to the upper temperature limit for the species) that were revealed by modern chromatographic and spectrometric methods.

Baicalospongia bacillifera is an endemic large-sized mushroom-shaped sponge that is widespread in Lake Baikal at depths of 4 m and below. The sponges were collected from a depth of 15 m in the southern part of the lake in August. The water temperature at the moment of sampling was 4°C.

A living sponge colony was adapted for 14 days to the artificial conditions of glass aquariums with flowing Baikal water at 4.5 and 10.5°C and 12-h photoperiod, without additional feeding. The water to the aquariums was supplied from a homemade refrigeration unit. After adaptation, sponge samples were taken for biochemical analysis by the methods described previously (Glyzina and Glyzin, 2014). Part of this work was performed using an Agilent 6890 gas chromatograph coupled with an MSD 5973N quadrupole mass spectrometer as a detector, DB-Wax columns with the inner diameter of 0.25 μm, and helium as a carrier gas (with a constant flow rate of 1.5 mL/min). Column temperature: 90°C (isotherm 4 min), 90–165°C (30°C/min), 165–225°C (3°C/min, isotherm 10.5 min); evaporator temperature, 250°C. Qualitative

Contents of dominant fatty acids and lipid groups in *Baicalospongia bacillifera*

Component of lipid fraction	Proportion relative to total lipids, %		Student's *t*-test*
	temperature conditions		
	4.5°C	10.5°C	
	fatty acids, % (relative to total amount of acids)		
14:0	2.17 ± 0.05	2.40 ± 0.03	3.945
16:0	7.05 ± 0.05	12.00 ± 0.06	63.378
i 17:0	0.11 ± 0.07	0.60 ± 0.07	5.950
17:0	1.53 ± 0.07	1.80 ± 0.07	2.727
i 15:0	0.38 ± 0.07	0.33 ± 0.04	0.620
ai 17:0	1.14 ± 0.07	1.45 ± 0.02	4.258
18:0	1.45 ± 0.07	0.51 ± 0.05	10.927
14:1 n-11	0.13 ± 0.07	0.49 ± 0.03	4.727
16:1 n-9	0.12 ± 0.07	1.13 ± 0.04	12.528
16:1 n-7	1.67 ± 0.07	0.92 ± 0.06	8.135
17:1 n-8	2.20 ± 0.07	5.35 ± 0.02	43.269
18:1 n-9	16.24 ± 0.02	25.50 ± 0.01	414.119
18:1 n-7	1.81 ± 0.05	0.14 ± 0.04	26.081
18:1 n-11	0.13 ± 0.03	0.52 ± 0.02	10.817
20:1 n-9	0.34 ± 0.02	1.23 ± 0.05	16.527
18:2 n-6	2.28 ± 0.01	6.45 ± 0.05	81.780
18:3 n-6	0.25 ± 0.02	0.4 ± 0.02	5.303
18:3 n-3	7.12 ± 0.04	10.3 ± 0.05	48.414
18:4 n-3	3.51 ± 0.01	3.14 ± 0.07	5.233
20:4 n-3	0.75 ± 0.05	0.44 ± 0.03	4.384
20:5 n-3	12.26 ± 0.02	7.18 ± 0.05	94.333
22:6 n-3	0.22 ± 0.04	0.12 ± 0.01	2.425
24:1 n-9	3.70 ± 0.01	2.94 ± 0.03	24.033
26:3 Δ5.9.19	14.65 ± 0.05	10.11 ± 0.05	64.205
∑PUFA	14.65 ± 0.05	10.11 ± 0.05	64.205
∑UFA	48.74 ± 0.02	38.69 ± 0.04	224.725
∑MUFA	9.91 ± 0.04	12.95 ± 0.07	37.707
∑aldehydes	6.32 ± 0.07	7.14 ± 0.08	7.714
∑sterols	4.28 ± 0.03	2.17 ± 0.07	27.706

* Differences are statistically significant ($p < 0.05$); number of degrees of freedom, 38; critical value of Student's *t*-test is 2.024 at significance level $\alpha = 0.05$.

analysis was based on comparisons of retention times and complete mass spectra with corresponding pure compounds, with reference to the NIST02.L data library and standard mixtures (Bacterial acid methyl esters CP Mix; Supelco, United States), as well as to the amount of the standard substance (deuteromethyl ether of tridecanoic acid) applied to the column. The analytical procedure allowed measurements with a relative error of ±10% at a 0.95 confidence probability within the entire range of test concentrations, with random error not exceeding ±5.2%.

As a result of biochemical analysis of lipid extract from *B. bacillifera*, 53 components of different chemical nature (including fatty acids, aldehydes, and sterols) were identified. Components of the lipid fraction with relative contents of more than 0.1% are listed in the table. Changes in the lipid components of cells in the sponge exposed at the critical temperature were limited the quantitative ratio of fatty acids, aldehydes, and sterols. During the experiments, we evaluated the composition of intracellular symbionts of sponges, the ratio of which depends primarily on the ambient temperature and concentration of organic substances in the water.

We found seven sterols with a total content of 47.0 rel. %. The major components of sterols are cholesterol (22.3 rel. %) and β-sitosterol (11.6 rel. %). It is known that cholesterol forces fatty acid residues to be arranged more densely manner and reduces their mobility and fluidity, thereby increasing microviscosity of cell membranes (Rod'kina et al., 2003).

It was found that the contents of some fatty acids (i 15:0) remain almost unchanged, whereas those of other such acids (16:0; 17:0) increase. This may be due