Symbiosis, 26 (1999) 179–191
Balaban, Philadelphia/Rehovot

Some Aspects of the Physiology and Biochemistry of *Lubomirska baikalensis*, a Sponge from Lake Baikal Containing Symbiotic Algae

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Received November 20, 1997; Accepted August 11, 1998

Abstract

We investigated the pigment content and carbon metabolism of *Lubomirska baikalensis*, a dominant fresh water sponge in Lake Baikal that contains symbiotic microalgae. The algae are morphologically similar to *Chlorella* spp. but lack chlorophyll *b*. Intact sponges from 15 m give a classical O_2 flux vs. irradiance response, but isolated algae respond with dramatically increased photosynthetic efficiency. Intact sponges from 28 m exhibit lower photosynthetic capacity and the isolated algae exhibit photoinhibition at higher irradiances. $^{14}CO_2$ is fixed via the C_3 pathway of photosynthetic carbon metabolism. A large fraction of the early products of photosynthesis accumulate as glycerate. Starch content increases with increasing habitat depth. The algae *in hospite* do not appear to adapt to decreased irradiance as depth increases.

Keywords: *Lubomirska baikalensis*, symbiotic algae

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0334-5114/99/$05.50

1. Introduction

There is modest information of the physiology and biochemistry of the more widely distributed fresh water sponges containing symbiotic algae (see Gilbert and Allen, 1973; Sand-Jensen and Pedersen, 1994) but very little is known of the sponges of Lake Baikal (Latyshev et al., 1992). The fresh water sponge *Lubomirska baikalensis* (Porifera; Lubomirskiidae) is one of a number of sponge species endemic to Lake Baikal, Siberia. *L. baikalensis* grows on solid substrates at depths of about 5 to 30 m, and appears as finger-like branches with a height of up to 50 cm. Sponge biomass can reach 5–10 kg/m^2.

In a previous study, Berner and Titlyanov (1992) determined that *L. baikalensis* contains about 10^9 unicellular algae per cm^2 of sponge surface, giving the sponge a bright green color. The algae are about 3 μm in diameter and reside, along with bacteria, in amoeboid archeocytes in a narrow layer no more than 3 mm from the surface of the sponge. The systematic position of both the algae and the bacteria, as well as their presumed role as intracellular symbionts, is unknown.

Despite the oligotrophic nature of Lake Baikal, observations on the architecture of the sponge, areal specific numbers of algae, and chlorophyll *a*. $cell^{-1}$, suggest that the algae in *L. baikalensis* are light limited over their 28 m bathymetric range (Berner and Titlyanov, 1992). The purpose of this study was to investigate basic aspects of photosynthesis and carbon metabolism in *L. baikalensis* from different depths to gain further insight into its ecophysiology.

2. Methods

Collection and maintenance of sponges

L. baikalensis was collected by divers from 5, 15, and 28 m near the Limnological Institute in Listvyanka, Siberia, on the southeastern shore of Lake Baikal in August 1990. Samples were brought to the laboratory within 30 minutes of collection and maintained at 12–13°C until needed. All sponge samples were used within a few hours of collection.

Isolation of algae

Algae were isolated according to the method of Latyshev et al. (1992). Briefly, sponges were squeezed in nylon mesh bags. The filtrate containing algae and particulate debris was concentrated by centrifugation in filtered lake water (FLW), washed several times, diluted with FLW, and layered on a

discontinuous Ficoll gradient (10, 5, 3, and 1%) using Ficoll 400 (Pharmacia, Uppsala, Sweden). Complete separation of algae from other particles was achieved in 24 hours at 10°C. The purity was checked by light microscopy. Ficoll was removed by washing the algae with FLW. The recovery ranged from 10^7–10^8 cells/ml.

Pigment extraction and chromatography

Pigment from intact sponges and from isolated algae was extracted in 90% acetone as described by Berner and Titlyanov (1992) and analyzed on a Phillips spectrophotometer using the equations of Jeffrey and Humphrey (1975).

Oxygen flux

Oxygen flux was determined by placing excised sections (0.5 cm diam; 0.5 cm length) of sponge branches or suspensions of isolated algae (each containing about 2×10^8 algae) in two ml of FLW in a water-jacketed glass respiration cell (Strathkelvin RC 300) fitted with a microcathode oxygen electrode (Strathkelvin SI 130) attached to an oxygen meter (Strathkelvin Model 781). The electrode was calibrated with FLW at 11°C. Calibration and measurements were recorded and analyzed with a microcomputer using Datacan software (Sable Systems). Temperature was maintained at 11–12°C by recirculating chilled water through the electrode chamber jacket. Irradiance was provided by a tungsten microscope lamp fitted with a diaphragm. Photosynthetically active radiation (PAR) was measured with a germanium photodiode sensor with linear characteristics (Titlyanov and Latypov, 1991) and recorded as flux density ($W.m^{-2}$). Irradiance was varied by changing the distance between source and sample. To compare intact sponges with isolated algae, oxygen flux data were expressed as $\mu l\ O_2.h^{-1}$ and normalized to 10^8 algal cells. Photosynthesis vs irradiance curves were fit using 50 iterations of a non-linear equation based on the Marquardt algorithm. Values for the maximum rate of net photosynthesis (P_{max}), the initial slope (α), compensation irradiance (I_c) and saturating irradiance (I_k) were derived from the curves.

$^{14}CO_2$ assimilation and products

To investigate photosynthetic products, small rectangular samples ($20 \times 12 \times 3$ mm) were excised from the sponge body wall just below a terminal osculum and allowed to adapt to saturating irradiance (300 $watts.m^{-2}$) in a small volume of FLW for 15–20 min at 22–24°C. Samples were then gently blotted to remove excess FLW and placed in a closed chamber in air containing $^{14}CO_2$ (540 mBq

mmol^{-1}). The total CO_2 was 0.6%. Samples were exposed for 30, 60, 300, and 600 seconds at saturating irradiance. Incubations were terminated by placing sponge samples in one ml of a boiling mixture of 80% ethanol and 5% formic acid.

To obtain products of heterotrophic fixation, sponge samples were treated similarly but dark adapted for 30 min and then incubated in darkness for 30 min with $^{14}CO_2$ (1.2% without dilution with $^{12}CO_2$) and then fixed in darkness in boiling ethanol:formic acid. Fixed samples were homogenized and centrifuged and the soluble supernatant fraction removed and prepared for analysis by two-dimensional paper chromatography using the method of Benson et al. (1951) with some modifications (Bil et al., 1991). Chromatograms were exposed to X-ray film (Kodak X-OMAT AR) for one month to locate radioactive products. Individual products were eluted from the chromatograms, counted by liquid scintillation (Beckman LS-100C), and expressed as ng-atom $^{14}C.cm^{-2}$ of sponge surface, and as the percentage of the total radioactivity of all assimilates on the chromatogram. The ethanol-formic acid insoluble fraction was treated sequentially with petroleum ether to recover lipids, and then a mixture of α- and β-amylase at 37°C to recover products of starch hydrolysis. These fractions and the remaining residual protein were sampled for scintillation counting.

3. Results and Discussion

Pigment analysis

Spectrophotometric analysis of a 90% acetone extract of crude sponge homogenate shows an absorption spectrum characteristic of chlorophyll *a* (peak absorption 665 nm) and *b* (indicated by the shoulder on the main peak) (Fig. 1, upper curve). However, a similar analysis of pigments extracted from isolated algae suggests that they lack chlorophyll *b* (Fig. 1, lower). The first derivative of the curve shows only one peak, consistent with this interpretation. Chlorophytes which contain only chlorophyll *a* belong to the Eustigmatophyceae (Hibberd, 1981).

However, the ultrastructure of algae belonging to this group is very different from that of the sponge symbionts. Except for the fact that no pyrenoid has been observed in the sponge algae, they are very similar to *Chlorella* spp., which is common as an endosymbiont in other freshwater sponges and in *Hydra* sp. (Gilbert and Allen, 1973; Wilkinson, 1987, 1992; McAuley, 1994). The chlorophyll *b* in the whole sponge extract is probably from epiphytic green algae but none were detected by light microscopy of sponge fractions from a Ficoll 400 gradient.

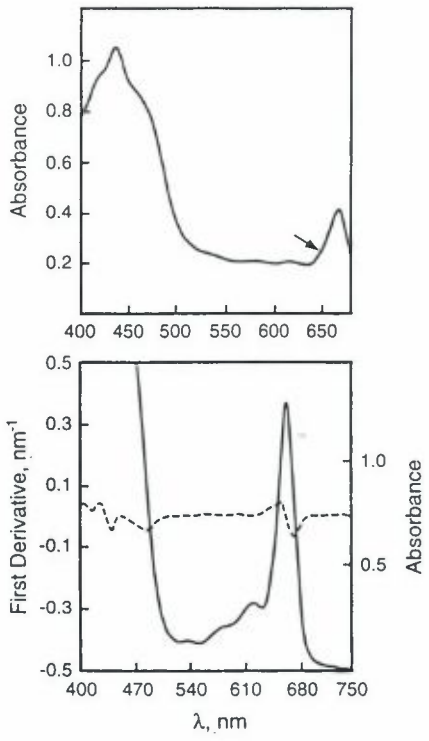

Figure 1. Upper, absorption spectrum of a 90% acetone extract of a crude suspension of algae obtained by squeezing a whole sponge. Chlorophyll *b* shoulder at arrow. Lower, absorption spectrum (solid line) and its first derivative of the curve (dashed line) of a 90% acetone extract of algae purified on a Ficoll gradient.

Oxygen flux

The time available at Lake Baikal was sufficient for only one series of net oxygen flux measurements at each of two depths (Fig. 2) and so the interpretation of these data must be regarded as tentative. However, they are in close agreement with those of Sand-Jensen and Pedersen (1994) for *Spongilla lacustris* from Denmark. Classical photosynthesis vs irradiance curves are shown in Fig. 2 for intact sponges and isolated algae from 15 and 28 m. At 15 m, intact sponge and isolated algae show the same P_{max} but the latter show dramatically increased photosynthetic efficiency, as indicated by α, and the concomitantly lower values for I_k and I_c (Table 1). At 28 m, P_{max} is lower in both sponges and isolated algae, compared to those at 15 m, indicating a light limitation (Fig. 2B). Again, isolated algae manifest a higher photosynthetic efficiency than the intact sponges (Table 1). Moreover, isolated algae from

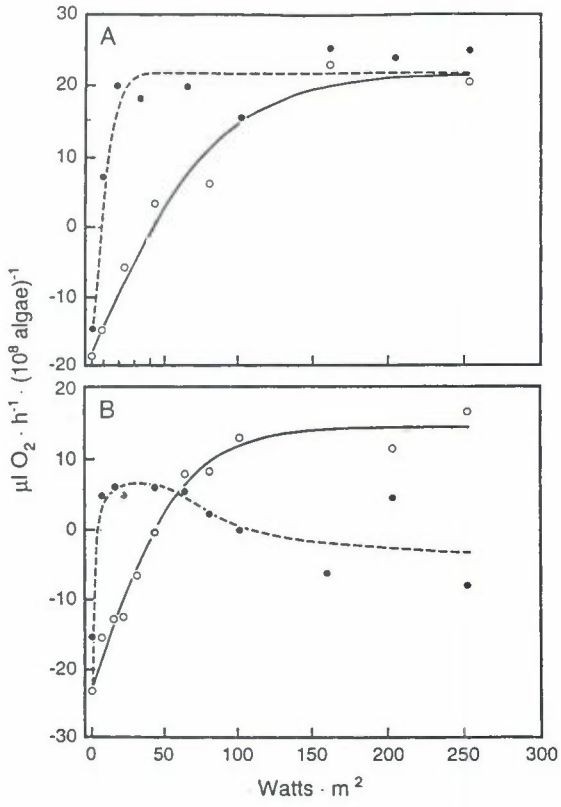

Figure 2. Photosynthesis vs irradiance curves for intact sponges (o) and isolated algae (•) from 15 m (A) and 28 m (B).

28 m, but not from 15 m, show clear evidence of photoinhibition at irradiances above 50 $W.m^{-2}$. The intact sponges need more light than the isolated algae to reach compensation and saturation. We speculate that the lower photosynthetic efficiency of algae in intact sponges, compared to isolated algae, is due both to light attenuation by the animal tissues and to self shading by the densely packed algae. A similar interpretation was drawn by Sand-Jensen and Pedersen (1994). Dark respiration ranged from 18–23 µl O_2.hr.10^8 cells in intact sponges and 14–16 µl O_2.hr.10^8 cells in isolated algae. These data suggest that a substantial fraction of the sponge respiration is due to the algae, as also noted in *S. lacustris* by Sand-Jensen and Pedersen (1994). As α, I_c and I_k did not change significantly with increasing depth, the algae from *L. baikalensis* are probably shade adapted. A similar conclusion was drawn by Berner and Titlyanov (1992) from ultrastructure of the algae. Photoacclimation to decreased irradiance by marine symbiotic microalgae can often, though not

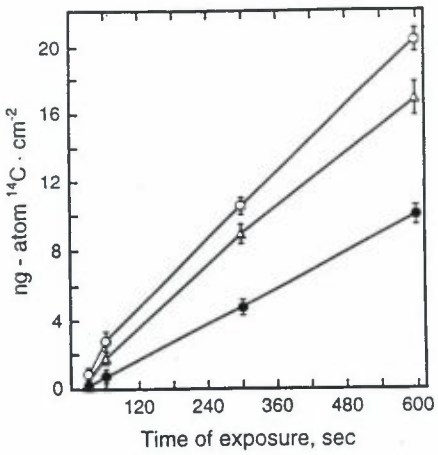

Figure 3. Total accumulation of carbon-14 during photosynthesis by rectangular explants of *L. baikalensis* from 5 (o), 15 (Δ), and 28 m (●). Sponge samples were pre-incubated at saturating irradiance at 22°C for 15 min in a small volume of FLW. They were then blotted and transferred to a closed chamber for incubation in air containing $^{14}CO_2$ (0.6%); 540 Mbq mmol^{-1}.

always, be accompanied by increased concentration of photosynthetic pigments, and by increased density of chloroplast thylakoids (Titlyanov et al., 1987). For example, symbiotic dinoflagellates in corals increase pigment content as habitat irradiance decreases (Titlyanov et al., 1980; Falkowski and Dubinsky, 1981; Dustan, 1982; Porter et al., 1984). The increased chlorophyll content results in significant changes in photokinetic parameters (Zvalinsky et al., 1980; Lelletkin and Zvalinsky, 1982). Unfortunately, no data are available on photoacclimation of symbiotic Chlorella in sponges (Wilkinson, 1987, 1992; Hill, 1996) or hydra (McAuley, 1994).

^{14}C assimilation and products

Accumulation of ^{14}C by intact sponges in the light is linear, and highest in shallow water sponges (Fig. 3). Rates of fixation, normalized to sponge surface area and algal cell number, decrease substantially as habitat depth increases, but assimilation numbers (rates normalized to chlorophyll *a*) decrease by only 10% (Table 1). This is not unexpected as the decrease in ^{14}C fixation in sponges from 28 m is concomitant with an almost 50% decrease in chlorophyll a per cell (Berner and Titlyanov, 1992). These data suggest that the algae tend not to exhibit photoacclimation with increasing depth.

Algae in *L. baikalensis* from all depths exhibit the typical C_3 pathway of carbon metabolism, and synthesize similar primary products of photosynthesis. During short term photosynthesis (30–60 sec), radioactive carbon in the water-soluble fraction is incorporated initially into 3-phosphoglyceric acid (3-PGA) and sugar phosphates (PES). With longer exposure to $^{14}CO_2$ (300–600 sec), label appears in fructose, glucose and sucrose. Assimilates typical of C_4 metabolism, such as malate and aspartic acid, do not exceed 10% of the total water soluble radioactivity (Table 3). Unexpectedly, after 30 seconds, and independent of habitat depth, about 40% of the radioactivity in the water-soluble fraction accumulates as glycerate and then abruptly decreases with time. Analysis of the data in Table 2 suggests that glycerate, which originates from 3-PGA, may be metabolized to amino acids, perhaps through serine and into glycine (the reverse of photorespiration), or by release from algae and metabolism by animal cells. ^{14}C accumulated in starch and protein after 60 sec (Table 4) and into lipid after 10 minutes (data not shown). Starch tended to increase in proportion to increasing habitat depth. The reason for this is obscure but may derive from some adaptation related to lower habitat temperature.

Table 1. Photosynthetic parameters of intact sponges and isolated algae

	Intact sponges		Isolated algae	
Depth (m)	15	28	15	28
P_{max} ($\mu l\ O_2\ hr^{-1}\ 10^8$ cells)	22	14	22	7
α ($\mu l\ O_2\ hr^{-1}\ 10^8$ cells^{-1} $(Wm^{-2})^{-1}$	0.16	0.18	2	<2
I_k (Wm^{-2})	90–92	72	10	<10
I_c (Wm^{-2})	40	40	<10	<10

Table 2. Rate of photosynthetic assimilation of $^{14}CO_2$ by *L. baikalensis* from 5, 15, and 28 m (X + S.D.; n = 3)

Depth (m)	^{14}C fixed ng-atom $cm^{-2}\ hr^{-1}$	ng-atom $(10^9$ algae$)^{-1}\ hr^{-1}$	g-atom (μg chl *a*)$^{-1}\ hr^{-1}$
5	130.0±6.0	127.0±6.0	2.8±0.7
15	107.0±5.0	nm	nm
28	61.0±3.0	65.0±3.0	2.5±0.5

nm = not measured.

Table 3. Primary ^{14}C-labelled products of photosynthesis in the symbiotic sponge *L. baikalensis* from 5, 15, and 28 m

Time (sec)	Percentage of total ^{14}C as *PGA+PES	Sugars	Aspartate	Malate	*PEP	Alanine	Serine	Glycine	Glutamate	Glycerate	Unk.
5 m											
30	4.0	10.0	1.3	3.9	0.8	9.3	17.5	4.5	0.5	38.9	9.3
60	22.2	17.6	2.0	8.5	1.6	15.3	9.3	11.1	1.0	5.0	6.4
300	14.8	28.2	4.0	4.9	1.2	17.1	3.7	18.4	1.1	1.7	4.9
600	7.0	32.1	5.5	3.3	0.3	25.7	5.7	11.0	1.2	0.5	7.7
15 m											
30	18.4	–	1.6	3.1	–	6.1	19.1	4.8	–	39.9	7.0
60	32.0	13.0	3.4	0.6	1.3	16.0	6.2	10.8	1.5	5.2	10.0
300	21.1	16.8	7.9	6.5	1.6	17.6	4.1	11.5	2.0	1.0	9.9
600	13.2	20.8	10.0	5.5	1.8	21.8	3.9	8.4	3.0	0.2	11.4
28 m											
30	21.7	–	2.1	2.9	0.7	4.9	20.6	5.6	–	30.9	10.6
60	34.6	3.3	4.5	14.5	1.8	16.6	4.2	10.8	–	3.0	6.7
300	22.2	19.7	4.4	6.8	1.8	18.1	2.0	14.2	1.5	1.2	8.1
600	17.0	20.9	8.3	8.1	2.6	17.4	3.4	7.6	2.7	0.5	11.5

*PGA = 3-phosphoglyceric acid; PES = phosphoric ethers of sugars; PEP = phosphoenolpyruvate and pyruvate. Relative deviation: for values more than 10, 5–10%; less than 10, 15–20%.

Table 4. Dynamics of accumulation of ^{14}C-labelled photosynthetic products of *L. baikalensis* from 5, 15, and 28 m.

Time (sec)	Photosynthetic products, ng-atom ^{14}C cm^{-2}									
	*PGA+PES	Sugars	Aspartate + malate	*PEP	Alanine	Serine + glycine	Glutamate	Glycerate	Starch	Protein
5 m										
30	0.04	0.10	0.05	0.01	0.09	0.20	0.01	0.37	nm	nm
60	0.55	0.44	0.26	0.04	0.38	0.50	0.02	0.12	0.05	0.01
300	1.56	2.97	0.94	0.13	1.80	2.33	0.12	0.17	0.20	0.08
600	1.47	6.74	1.85	0.23	5.40	3.51	0.25	0.10	0.50	0.19
15 m										
30	0.14	–	0.05	–	0.05	0.18	–	0.30	nm	nm
60	0.61	0.25	0.07	0.02	0.30	0.32	0.03	0.10	0.04	0.01
300	1.90	1.51	1.29	0.14	1.58	1.40	0.18	0.09	0.78	0.09
600	2.35	3.71	2.76	0.32	3.88	1.86	0.53	0.03	3.0	0.19
28 m										
30	0.08	–	0.02	0.01	0.02	0.10	–	0.12	nm	nm
60	0.33	0.03	0.18	0.02	0.16	0.14	–	0.03	0.02	0.01
300	1.72	0.97	0.54	0.09	0.90	0.80	0.07	0.06	0.30	0.05
600	1.74	2.14	1.68	0.27	1.78	1.07	0.28	0.05	1.02	0.11

*PGA = 3-phosphoglyceric acid; PES = phosphoric ethers of sugars; PEP = phosphoenolpyruvate and pyruvate. Relative deviation: for values more than 10, 5–10%; less than 10, 15–20%; nm = not measured.

Table 5. Heterotrophic $^{14}CO_2$ fixation by *L. baikalensis*

Depth (m)	5	15	28
^{14}C fixed (ng-atom ^{14}C cm^{-2} h^{-1})	1.90±0.1	1.86±0.1	1.38±0.06
Compounds labelled (ng-atom ^{14}C cm^{-2})			
PGA+PES	0.04	0.13	0.10
Sugars	0.05	0.02	0.01
Aspartate	0.17	0.19	0.11
Malate	0.11	0.16	0.19
Alanine	0.03	0.04	0.01
Serine	0.03	0.01	0.03
Glycine	0.02	0.02	0.03
Glutamate	0.21	0.17	0.09
Krebs cycle acids	0.03	0.03	0.03
Pigments	0.04	0.03	0.04
Unknown	0.21	0.14	0.07

PGA = 3-phosphoglyceric acid; PES = phosphoric ethers of sugars.

Heterotrophic fixation of $^{14}CO_2$

L. baikalensis fixed $^{14}CO_2$ heterotrophically at 1.5 to 2.3% of the rate of photosynthetic fixation. The rate decreased 1.4-fold from 5 m to 28 m. It is likely that not only miocroalgae but also sponge cells and associated microflora and fauna contributed to heterotrophic fixation. ^{14}C accumulated mainly in aspartate, malate, and glutamate. The ratio aspartate : malate decreased from 1.55 at 5 m to 0.58 at 28 m. The predominance of C_4 acids suggests that heterotrophic fixation of CO_2 takes place via activity of phosphoenol-pyruvate carboxylase.

4. Conclusions

The unidentified symbiotic microalgae of *L. baikalensis* lack chlorophyll *b*. They do not appear to adapt to decreased irradiance associated with increased depth. Carbon is fixed in photosynthesis via the C_3 pathway. A large fraction of the early products of photosynthesis derive from the dephosphorylation of 3-phosphoglyceric acid.

Acknowledgements

This investigation was supported by the Institute of Marine Biology, Vladivostok, Russia. We thank Dr. N. Latishev for help with laboratory and field work and Dr. M. Grachev, Director, Limnological Institute, Listvyanka, for generous provision of facilities and support.

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