Light-enhanced survival of *Dinoroseobacter shibae* during long-term starvation

**Introduction**

*Dinoroseobacter shibae* is an aerobic anoxygenic phototroph (AAP), capable of using light as an additional energy source under anoxic conditions. While light is known to increase growth yields, the cells do not grow by light energy alone (1). We suspected that the cells benefit from light particularly under conditions of carbon and electron donor limitation.

To test this, long-term starvation experiments were performed with cells grown in both complex marine broth and defined minimal medium with succinate as the only substrate. The cells were incubated under three different conditions, i.e. continuous darkness (DD), continuous light (LL), and a day-night cycle (LD, 12 h/12 h).

To assess viability and the energetic state, several biomass parameters (total and live count, dry mass, pigment and ATP content) and activities (respiration rates, light- and respiration-driven proton translocation) were analysed.

**Fig. 1:** *Dinoroseobacter shibae* showing their characteristic pigmentation.

**Fig. 2:** Experimental set-up to find the optimum light intensity for survival

**Results**

**Morphological changes of cells during long term starvation**

![Morphological changes](image)

**Fig. 3:** Changes in total cell and viable counts of *D. shibae* under different incubation conditions during long term starvation

**Fig. 4:** Morphological changes analysed by transmission electron microscopy (TEM). Freshly grown *D. shibae* (a) and (b) under light/dark cycle (LD, 12/12h) were regular shaped and flagellated. DD cells (c) were irregular, whereas the LD cells still possessing their flagella (d) after three weeks of starvation.

![Morphological changes](image)

**Fig. 5:** Detection of polyhydroxyalkanoates (PHA s) by Nile blue staining. Initial stationary phase (a) and three weeks starved (b) LD cells showed the presence of PHA s.

**Physiological fitness of cells during long term starvation**

![Physiological fitness](image)

**Fig. 6:** Number of protons translocated in (a) light (LL) and (b) light/dark (LD) starved cell suspensions of *D. shibae* per added oxygen atom (H₂O).

**Table 1:** Cytoplasmic ATP concentrations under light (LL, grey colored) and light/dark (LD, orange colored) starved cell suspensions of *Dinoroseobacter shibae*. The washed cells were incubated under anoxic, with oxygen and additional light and only with succinate (10mM).

<table>
<thead>
<tr>
<th>Days Starved</th>
<th>mmol ATP I in the cytoplasm</th>
<th>mmol ATP II in the cytoplasm</th>
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<tbody>
<tr>
<td></td>
<td>LL</td>
<td>LL + O₂</td>
</tr>
<tr>
<td>3</td>
<td>13a±0.2</td>
<td>0.01±0.2</td>
</tr>
<tr>
<td>7</td>
<td>7.9±0.2</td>
<td>0.03±0.2</td>
</tr>
<tr>
<td>14</td>
<td>3.3±0.2</td>
<td>0.05±0.2</td>
</tr>
<tr>
<td>21</td>
<td>0.28±0.2</td>
<td>0.01±0.2</td>
</tr>
</tbody>
</table>

**Conclusions**

In general, cells incubated under medium light intensity (12 µE m⁻² s⁻¹) showed the highest survival rates. At high light intensity the bacteriochlorophyll a concentrations decreased, while the carotenoids increased, indicating their photo-protective function (Fig. 2).

After four weeks of the starvation

— LD cells had 10 fold higher total cell counts and also increased viable counts (Fig. 3).
— Both LL and DD cells looked irregular. LD cells were less irregularly shaped, and some were still flagellated (Fig. 4).
— Nile blue staining showed that the (PHA) were still detected in LD cells (Fig. 5)
— With LD cells, there was not much light effect in the beginning, but at the end protons were translocated by light (Fig. 6).
— The cytoplasmic ATP tends to decrease, but seems to higher in the LD than in the dark (Tab. 1)

Our study shows that the cells benefit from the light and dark rhythm during starvation.

**References:**