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# Growth and bacteriochlorophyll *a* formation in taxonomically diverse aerobic anoxygenic phototrophic bacteria in chemostat culture: Influence of light regimen and starvation

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This article is dedicated to Prof. Deckwer on the occasion of his 65th birthday

**Abstract**

The influence of light and starvation on bacteriochlorophyll *a* (bchl *a*) and biomass formation of some aerobic anoxygenic photosynthetic bacteria was investigated in chemostat culture. Three species recently isolated from dinoflagellate cultures were compared, *Dinoroseobacter shibae*, which contained relatively high amounts of bchl *a*, and *Stappia* sp. DFL-11 and *Hoeflea phototrophica*, which both have very low amounts of photosynthetic pigments. Simulating day/night alternation *D. shibae* was subjected to 7 light:dark cycles = 8 h:16 h and 3 light:dark cycles = 16 h:8 h. Using a dilution rate of 0.1 $h^{-1}$ and succinate as a substrate (8.5 mmol/l) the concentration of bchl *a* decreased during the light period due to inhibition by light and recovered always to about the previous level during the dark period. Biomass increased during the light period showing that additional energy was generated in the light.

Nutrient deficiency in the absence or presence of light was studied by exposing continuous cultures growing in the dark under the conditions described above to 8 h of illumination, starvation, i.e. interruption of medium supply, or both. The cultures of *D. shibae* exhibited a bchl *a* base level of 2.5 nmol/mg protein, which decreased reversibly in the light and increased significantly during starvation in the dark to reach a level of 4 nmol/mg protein 16 h after medium supply was resumed, indicating a slow regulatory response towards periodic starvation in *D. shibae*. Under simultaneous illumination and starvation conditions, these two effects apparently cancelled one another out, resulting in unchanged levels of bchl *a*. By contrast, the cultures of *Stappia* sp. and *H. phototropica* were characterized by bchl *a* contents of almost zero in the dark, little change under illumination or starvation, but a very marked increase after simultaneous illumination and starvation, reaching 0.16 and 0.24 nmol bchl *a*/mg protein, respectively, 16 h after medium supply was resumed. These data suggest the presence of a regulatory mechanism integrating light stimuli and starvation related metabolic signals. The different physiological behaviour of the two groups is discussed in terms of the significance of aerobic anoxygenic photosynthesis in their natural environment.


*Keywords:* Aerobic anoxygenic phototrophic bacteria; Chemostat; Diurnal cycle; Starvation; Light

## 1. Introduction

The aerobic anoxygenic photosynthetic bacteria (AAnPs) possess a perfect photosynthetic apparatus including light harvesting systems, reaction center, bacteriochlorophyll *a* (bchl *a*) and carotenoids just as their anaerobic counterparts, the purple nonsulfur bacteria. But whereas the purple bacteria either grow photoheterotrophically under anaerobic conditions or chemoheterotrophically under aerobic conditions, both types of energy generation are active at the same time in the AAnPs. The AAnPs are strict aerobes (with the exception of some strains, e.g. *Roseobacter denitrificans* and *D. shibae*, which are able to use nitrate as an electron acceptor) and, although mechanistically of the anoxygenic type, photosynthesis proceeds under aerobic conditions. Paradoxically the photsynthetic apparatus is synthesized only in the dark and synthesis ceases as soon as light has admission. This seems to restrict the use of light energy, since the photosynthetic complex that is left over from a preceding dark period is gradually diluted by growth. However, in nature with its

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diurnal light and dark rhythm this does not really constitute a problem as doubling rates per day are small, and thus the photosystem can be restored every night and is available in the morning. There are indications that supply of organic nutrients has to be limited for photosynthetic pigment formation, suggesting that supplementary energy generation by light would render the AAnPs a selective advantage especially under the oligotrophic conditions of the open ocean (see [1] for more background).

The benefit of light periods on growth of AAnPs was first shown by Harashima et al. [2] using *R. denitrificans*. Batch cultures that were grown in the light after a 24 h dark period yielded a cell protein content twice as high as cultures that were grown permanently in the dark or in the light, and the growth rate also increased by a factor of two. Recently Kolber et al. [3] obtained a fivefold higher biomass under a day/night cycle relative to permanently dark conditions with a marine *Erythrobacter* isolate, and a 20–40% higher growth rate. Results that were closer to natural conditions were presented by Yurkov and van Gemerden [4,5] using chemostat cultures of *Erythromicrobium hydrolyticum*. Under a light regimen of 14 h light and 10 h darkness the protein level was 11% higher at the end of the light period and 4% at the end of the dark period relative to permanently dark conditions.

Inhibition of bchl *a* formation by light has been unanimously confirmed by all authors concerned [2,4,6–8]. Very low light intensities were sufficient, i.e. half maximum inhibition occurred at 1.2 $W/m^2$ for *R. denitrificans* [9]. Only from methylotrophic AAnPs there are early reports of a positive action of light on pigment formation [10]. Although these organisms were also not able to form bchl *a* in the light, their bchl *a* content was enhanced if a light period was applied during the first part of the logarithmic growth phase and was then up to three times higher than in a culture grown in complete darkness. Shimada [11] spoke of light as a positive effector for bchl *a* synthesis. Unfortunately this interesting observation has never been verified and further pursued up to now. It could be a starting point for investigations in the area of signal transduction.

The necessity of nutrient deficiency for pigment formation in aerobic anoxygenic phototrophic bacteria is highly plausible, but not well documented in the literature. It was shown that photosynthetic pigments were not formed if cultures were transferred to a rich medium or to a medium with a readily available carbon source [2,3,12]. The chemostat experiments of Yurkov and van Gemerden [4] implied substrate limiting conditions *per se*, but as shown by Suyama et al. [13] for *Roseateles depolymerans* complete absence of nutrients, i.e. periods of starvation, may be required for triggering formation of the photosynthetic complex.

We have isolated a number of marine strains that were shown to harbor the *pufL* and *pufM* genes which code for proteins of the photosynthesis reaction center [14]. One group of these strains, recently described as *Dinoroseobacter shibae* [15], was always distinctly red or pink pigmented, if grown in the dark. Two other phylogenetic groups, i.e. *Hoeflea phototrophica* [16] and *Roseovarius mucosus* [17] contained very small amounts of bchl *a* depending on the growth conditions, another group (*Stappia* sp. DFL-11, Biebl et al., unpublished) contained only traces, and in the fifth group photosynthetic pigments could not be found. Here we studied the influence of light and nutrient deficiency, singly and combined, on cell mass and bchl *a* formation in chemostat cultures in representative strains of the above groups disregarding the one without bchl *a*. Of special interest were the low pigment groups as the bchl *a* amounts hitherto detected are obviously not sufficient to provide an appreciable contribution to energy generation, and it appears possible that these organisms require conditions more relevant to their natural environment to initiate formation of the photosynthetic apparatus. Therefore nutrient deficiency was not only investigated under substrate limitation typical of chemostat culture, but also by applying periods of total starvation. In addition a series of day/night cycles was investigated for *D. shibae* both under a short day/long night and a long day/short night regimen.

## 2. Methods

The *strains* used in this investigation were isolated from dinoflagellate cultures as described by Allgaier et al. [14]: *D. shibae* DFL-12 (=DSM 16493 = NCIMB14021) [15], *Stappia* sp. DFL-11 (=DSM 17067 = MCIMB 14079) (Biebl et al., unpublished), *H. phototrophica* DFL-43 (=DSM 17068 = NCIMB 14078) [16], and *R. mucosus* DFL-24 (=DSM 17068 = NCIMB 14077) [17]. They were maintained and precultured in a medium containing 20 g sea salts (Sigma), 3 g Bacto peptone (Difco), and 0.5 g yeast extract per liter of distilled water.

The *continuous cultures* were run in a 1 l glass fermenter with a water coat for temperature control using a working volume of 0.5 l. The culture was stirred at a rate of 120 rounds/min and aerated with a flow of 40 ml/min corresponding to 0.08 vvm. Temperature was maintained at 30 °C by a cryostat to lead the heat produced by the lamps away. For illumination three 60 W krypton incandescent lamps (Osram) were mounted at a distance of 8 cm from the fermentor wall and dimmed electronically to 60% intensity. The resulting illuminance measured at the fermenter wall was 1400 lx (400–700 nm), corresponding to about 30 $\mu E\ m^2\ s^{-1}$.

The culture medium consisted of 20 g Sigma sea salts, 0.3 g $(NH_4)_2SO_4$, 0.2 g $KH_2PO_4$ 1.37 g di-Na-succinate, 0.1 g yeast extract, 1 ml of trace element solution SL12 [18] and 1 ml 1 M $H_2SO_4$ per litre. For strain DFL-43, which required complex nutrients and lower salinity, 10 g sea salts, 2 g Bacto peptone, 0.5 g yeast extract, 1 ml SL 12 and 0.15 ml 1 M NaOH were used. The pH stably adjusted itself to 8.0–8.2 and was not regulated. The medium was supplied and the culture fluid was removed by peristaltic pumps adjusted to obtain a dilution rate of 0.1 (±0.02) $h^{-1}$. The bioreactor was autoclaved and inoculated with 10% of a dark grown batch culture. Steady state was reached after approximately four residence times.

### 2.1. Analytical methods

Cell density was measured as optical density at 650 nm. Cell protein was determined using the bicinchoninic acid (BCA) method [19] as supplied by the Pierce company (Rockford, Illinois, USA). In the variation applied (“micro-BCA”) cell desintregration by chemical lysis and analysis were performed in one step. 0.1 ml of a culture having an $OD_{650}$ of 0.5–1.0 was washed in PBS buffer. The pellet was suspended in 1 ml of distilled water, and 0.5 ml of this suspension was mixed with the alkaline BCA reagent (0.1 M NaOH) in a 1.5 ml Eppendorf tube. The small amount of cells present was completely dissolved by the NaOH. The mixture was kept at 60 °C for 1 h and then cooled to room temperature. Its absorption was measured at an OD of 562 nm within 10 min. For calibration, serum albumin V (Roth, Karlsruhe, Germany) was used in a concentration range from 6 to 24 mg/l. In one experiment (day/night