Growth of the phototrophic purple sulfur bacterium *Thiocapsa roseopersicina* under oxic/anoxic regimens in the light

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1. SUMMARY

The phototrophic purple sulfur bacterium *Thiocapsa roseopersicina* was grown in sulfide-limited continuous cultures exposed to oxic/anoxic regimens in continuous light. Synthesis of bacteriochlorophyll *a* (BChla) did not occur during the oxic periods, but started immediately upon the creation of anoxic conditions. In contrast, protein synthesis continued during both oxic and anoxic periods. Consequently, the specific content of BChla fluctuated. Despite the presence of oxygen and the fluctuating BChla content, growth occurred predominantly in a phototrophic mode and respiration was virtually zero.

BChla synthesis continued at high rates during anoxic periods, thus compensating for the lack of synthesis during oxic periods. When cultivated under regimens with oxic periods longer than 12 h the specific BChla content was always lower than 27 μg·mg⁻¹ protein and decreased with increasing length of the oxic periods. During the anoxic periods, BChla synthesis occurred at the maximal velocity of 1.2 μg·mg⁻¹ protein·h⁻¹, but the length of the anoxic periods was not sufficient to allow the BChla content to reach the maximum level.

Cultivation under continuously oxic conditions eventually resulted in pigmenless cells growing chemolithotrophically. The BChla synthesizing ability was not lost during prolonged exposure to oxygen.

It was concluded that *T. roseopersicina* is very well adapted to oxic/anoxic cycles.

2. INTRODUCTION

Laboratory studies have shown that under oxic conditions pigment synthesis is inhibited in purple sulfur bacteria including *T. roseopersicina* [1,2]. For *Chromatium vinosum* it was demonstrated that the cells remained viable for at least 12 h [3]. In addition, it has been demonstrated unequivocally that many species of purple sulfur bacteria includ-
ing *T. roseopersicina* are able to grow chemolithotrophically in the dark in the presence of oxygen and an appropriate electron donor [1,2, 4–8]. *T. roseopersicina* is also able to photosynthesize under anoxic conditions provided that the cells contain BChl *a* [2].

The aim of the present study was to investigate growth and pigment synthesis of illuminated *T. roseopersicina* cultures during oxic/anoxic regimens and the occurrence of photosynthesis and chemosynthesis under these conditions.

3. MATERIAL AND METHODS

3.1. Bacterial strain

All experiments were performed with *T. roseopersicina* strain M1, isolated from a laminated microbial ecosystem on the island of Mellum (F.R.G.).

3.2. Growth conditions and procedures

*T. roseopersicina* was cultivated in modified ASN-III medium [2]. Growth experiments were performed using sulfide-limited continuous cultures as described by Beekink and Van Gemerden [9]. All experiments were carried out at a light intensity of 110 µE. m⁻². sec⁻¹ (incandescent lig.), temperature 25°C, a. j pH 8.0 (pH-stat). The dilution rate (D) was maintained at 0.03 h⁻¹.

Cultures were subjected to different oxic/anoxic regimens with a total time span of 24 h. The oxygen concentration was monitored with a custom-made cathode type electrode. During the oxic periods the oxygen concentration was maintained at 60 ± 10 µM by a time-controlled intermittent supply of air. When the air supply was interrupted (time switch) the O₂ concentration decreased linearly to zero in the course of 2 h 45 min. At the end of the anoxic periods the supply of air was restored and constant O₂ concentrations were reached after 4 min. Steady states cannot be achieved when organisms are cultivated under fluctuating conditions. Nevertheless, a time-dependent dynamic equilibrium was reached after prolonged cultivation under all regimens. For comparison *T. roseopersicina* was also cultivated under continuously anoxic conditions.

During cultivation under continuously oxic conditions the oxygen concentration was monitored with a Yellow-Springs oxygen electrode connected to a custom-made polarographic measuring and controlling device (oxygen titrator). Oxygen regulation was performed by a controlled pulsing of air into the culture (0.1 bar pressure at a flow-rate of 10 l. h⁻¹). The oxygen concentration during the steady state was approximately 70 µM.

The rate of oxygen disappearance was determined from the linear decrease after a pulse of oxygen had been supplied to the culture. The abiotic oxygen consumption was determined in media of the same composition containing 40 µM sulfide. The specific oxygen uptake rate (qO₂) was calculated from the difference between the oxygen disappearance rate and the abiotic oxygen consumption rate.

The ability of cells grown under continuously oxic conditions to synthesize BChl *a* was studied in batch cultures supplied with sulfide.

Sulfide was measured with the methylene-blue method [10], or with ion-specific electrodes when the concentration was below 0.2 mM [11].

The refractile intracellular globules in Chromatiaceae in the past have been considered to be elemental sulfur. Data on the buoyant density of the globules indicate that a less dense compound is included as well [12,13]. It is conceivable that the sulfur is present as long-chain polythionates [14,15]. Therefore, use of the term 'elemental sulfur' is avoided and the sulfur species inside the globules is referred to as zero valence sulfur (S⁰). The compound is easily extracted in most organic solvents. BChl *a* and S⁰ were determined in methanol extracts of whole cells according to Stal et al. [16]. Protein was determined using the Folin phenol reagent [17], after extraction of S⁰ and BChl *a* and subsequent solubilization of the pellet in 1 M NaOH at 100°C [18]. Total sugar concentrations were determined using the anthrone reagent [19] and glycogen was calculated according to [20]. Poly-β-hydroxybutyrate (PHB) was determined according to [21].

In order to calculate the redox balance, all electron consuming and donating reactions were normalized to specific rates using protein as measure of cell material, thus allowing the addition of
all specific rates. Protein concentrations obtained in phototrophically outgrown batch cultures were correlated with the quantity of electrons required for the synthesis of cell material by using a conversion factor \( f_t \) determined as being 0.456 mmol e\(^-\)/mg protein. Glycogen concentrations were transposed to the quantity of reducing equivalents required for its synthesis using a conversion factor \( f_2 \), being 0.136 mmol e\(^-\)/mg glycogen monomer [20].

4. RESULTS

4.1. Cultivation under different oxic/anoxic regimens

*T. roseopersicina* was cultivated in sulfide-limited continuous cultures \((D = 0.03 \text{ h}^{-1}, \text{ continuous light})\) subjected to the following oxic/anoxic regimens (in hours) 4/20, 7.5/16.5, 12/12, 16/8 and 21/3.

The time course of the concentrations of protein and BChla in a culture subjected to a regimen of 12 h oxic/12 h anoxic for more than five volume changes is shown in Fig. 1. The sulfide concentration in the reservoir \((S_R)\) was 1.95 mM. During the oxic period the BChla concentration decreased according to the theoretical wash-out curve, whereas during the anoxic period an increase was observed. Growth occurred during both the anoxic and the oxic period, but unexpectedly at the highest rates during the oxic periods. Over a 24-h cycle growth was balanced by wash-out. The glycogen concentrations remained approximately constant at 3.5 mg·l\(^{-1}\). Poly-\(\beta\)-hydroxybutyrate was not detected. Sulfide concentrations fluctuated between 25 and 85 \(\mu\text{M}\), but the variations did not clearly coincide with the regimen employed.

The fluctuations in the concentrations of protein, BChla and glycogen during cultivation at a regimen of 21 h oxic/3 h anoxic were shown in Fig. 2. In this case the sulfide concentration in the reservoir \((S_R)\) was 3.96 mM. Again, the BChla concentration decreased during the oxic periods and increased during the anoxic periods. The protein and glycogen concentrations remained approximately constant. The sulfide concentration fluctuated between 32 and 77 \(\mu\text{M}\).

Owing to the fact that the fluctuations in BChla were more pronounced than and opposite to those of protein, the specific BChla content varied. In the cultures subjected to 12 h/12 h and the 21 h/3 h (oxic/anoxic) regimens the ranges were 21–29 \(\mu\text{g}·\text{mg}^{-1}\) protein and 4–7 \(\mu\text{g}·\text{mg}^{-1}\) protein, respectively.

Under all oxic/anoxic regimens it was observed that the BChla concentration fluctuated strongly, whereas the protein concentrations showed smaller fluctuations (Table 1). In contrast, in the continuously anoxic steady-state the BChla

![Fig. 1. Time course of BChla and protein concentrations in a continuously illuminated sulfide-limited culture of *T. roseopersicina* strain M1 (\(D = 0.03 \text{ h}^{-1}\)) after cultivation for five volume changes under a 12-h oxic/12-h anoxic regimen. Dashed lines indicate the effect of wash-out (i.e., synthesis rate equals zero).](https://academic.oup.com/femsec/article-abstract/6/1/69/860760)

![Fig. 2. Time course of BChla and protein concentrations in a continuously illuminated sulfide-limited culture of *T. roseopersicina* strain M1 (\(D = 0.03 \text{ h}^{-1}\)) after cultivation for five volume changes under a 21-h oxic/3-h anoxic regimen. Dashed lines indicate the effect of wash-out (i.e., synthesis rate equals zero).](https://academic.oup.com/femsec/article-abstract/6/1/69/860760)
Table 1

Concentrations of BChlα and protein, and the ratio of BChlα/protein in sulfide-limited cultures of *T. roseopersicina* strain M1 (∆ = 0.03 h⁻¹) subjected to oxic/anoxic regimens in continuous light. Vc is the average synthesis rate of BChlα during the anoxic periods.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Sf sulfide (mM)</th>
<th>End of anoxic period BChlα (μg·l⁻¹)</th>
<th>protein (mg·l⁻¹)</th>
<th>BChlα/prot. (μg·mg⁻¹)</th>
<th>End of oxic period BChlα (μg·l⁻¹)</th>
<th>protein (mg·l⁻¹)</th>
<th>BChlα/prot. (μg·mg⁻¹)</th>
<th>Vc (μg·mg⁻¹·h⁻¹)</th>
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<tbody>
<tr>
<td>+O₂/-O₂</td>
<td>(h) (h)</td>
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<td></td>
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<td></td>
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<td>29.1</td>
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<td></td>
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<td>880</td>
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<td>770</td>
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<tr>
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<td>3.70</td>
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<td></td>
<td>1.01 h</td>
</tr>
</tbody>
</table>

a Values were determined after cultivation for five volume changes under the regimens indicated.

b Determined in a shift experiment (see Fig. 4), during the steady state the continuous exposure to oxygen resulted in zero BChlα synthesis rate.

c The abiotic oxygen consumption was determined separately in media of identical composition but devoid of cells and was found to be 0.34 μmol O₂·l⁻¹·min⁻¹. For all oxic/anoxic regimens studied, the rate of oxygen disappearance in the culture was estimated to be 0.4 μmol O₂·l⁻¹·min⁻¹ indicating that respiration with oxygen virtually did not occur. In addition, it was demonstrated that endogenous respiration in the light was essentially zero. However, in continuously oxic cultures the oxygen disappearance rate was 1.34 μmol O₂·l⁻¹·min⁻¹, thus exceeding the abiotic oxygen consumption by a factor of four. The specific respiration rate (qO₂) was 58 nmol mg⁻¹ protein·min⁻¹.

In cultures subjected to oxic/anoxic regimens, the yield (protein/sulfide) was similar to that observed in continuously anoxically grown cultures. In contrast, in cultures growing under continuously oxic conditions the yield was much lower even when the occurrence of intermediate products (i.e. intracellular S⁰) and storage compounds (i.e. glycogen, PHB) was taken into account. Calculations were performed on the redox balance; the conversion factor between protein and cell material (f₁) was assumed to be constant (See Methods Section). In continuously anoxic cultures and in cultures subjected to oxic/anoxic regimens, most electrons were used for the synthesis of cell material; the loss of electrons due to abiotic

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Fig. 3. Redox calculations performed on *T. roseopersicina* strain M1 continuous cultures (∆ = 0.03 h⁻¹) subjected to various oxic/anoxic regimens. Bars indicate sulfide input and loss (I) and substrate recovered in the form of cell material (calculated from protein), glycogen, S⁰ and sulfide, respectively, at the end of both the anoxic (A) and oxic periods (B).

Data were collected after cultivation had proceeded for more than five volume changes at the regimen indicated.
reactions was 3–14% (Fig. 3). The contribution of glycogen, $S^0$ and residual sulfide was of only minor importance, and the total amount of electrons recovered in cell material (including storage polymers and $S^0$) almost equaled the input minus the contributions of abiotic reactions, thus indicating that virtually no electrons were consumed by oxygen respiration. As expected, for the chemolithotrophically growing culture a large discrepancy was observed between the input and the reducing equivalents recovered in cell material including storage polymers and $S^0$.

4.2. Shift from chemolithotrophy to photolithotrophy

In order to study whether oxically grown cells $(D = 0.03 \text{ h}^{-1})$ are able to synthesize BChla, a pigmentless culture in steady state (more than 10 volume changes oxic growth) was shifted to anoxic conditions. At zero time the oxygen supply and the medium supply were shut off. Oxygen was completely depleted after 40 min. At time $t = 1$ h sulfide was added to a final concentration of 1.1 mM. The concentrations of BChla, protein, $S^0$, and sulfide are shown in Fig. 4.

BChla synthesis started almost immediately upon exposure to anoxic conditions. The specific BChla-synthesis rate was $1 \mu g \cdot mg^{-1} \text{ protein} \cdot \text{h}^{-1}$, which is 81% of the maximum synthesis rate observed in oxic/anoxic regimens (see Table 1).

Temporarily, the oxidation of sulfide resulted in $S^0$ accumulation. Photooxidation of sulfide and $S^0$ resulted in growth and the final result was an outgrown culture (sulfide and $S^0$ completely oxidized to sulfate) with increased concentrations of protein and BChla.

5. DISCUSSION

This report demonstrates that *T. roseopersicina* strain M1 is able to grow under shifting oxic/anoxic conditions. During theoxic periods no synthesis of BChla occurred, whereas it started immediately upon the creation of anoxic conditions. However, growth under the shifting conditions was not restricted to the anoxic periods; protein synthesis took place during both the oxic and the anoxic periods. Consequently, the specific BChla content fluctuated during oxic/anoxic cycles; the lowest and highest values occurred at the end of oxic and anoxic periods, respectively. At present no explanation can be offered for the higher specific growth rates in the oxic periods. The observations suggest that the rate-limiting biosynthetic process is stimulated by oxygen.

*T. roseopersicina* is also able to grow in the continuous presence of oxygen [1,2,5,6]. Under these conditions BChla-synthesis does not occur and eventually the cells become devoid of photos pigments and grow chemolithotrophically. Nevertheless, even after continuous exposure to oxygen for 10 volume changes, the organism was still capable of synthesizing BChla when shifted to anoxic conditions (Fig. 4). The high specific rate $(k_c$, see Table 1) indicated that a large proportion of the cells were participating in the synthesis of BChla.

Pigmentless chemolithotrophically growing *T. roseopersicina* cells were actively respiring, using oxygen as the terminal electron acceptor. Under these conditions the protein concentration was approximately one third of that under continuously anoxic light conditions (see Table 1 and Fig. 3). Similar phenomena have been reported for *T. roseopersicina* when growing on thiosulfate [2].

In contrast, all cultures subjected to oxic/anoxic regimens contained BChla. Thus, in the
light, growth during the oxic periods may occur phototrophically, chemotrophically or both. Evidently, oxygen respiration did not occur to any extent, irrespective of the oxic/anoxic regimens studied, since the oxygen disappearance was due almost exclusively to the abiotic reactions of oxygen with sulfide. Secondly, it could be judged on the basis of cell density that light and not the aerobic oxidation of sulfide served as energy source. As indicated in Fig. 3, with oxic periods up to 21 h in a 24-h cycle the sulfide utilized could be completely accounted for as cell material, including glycogen and S°, at the end of both oxic and anoxic periods. These data indicate that no sulfide was respired with oxygen and that extracellular organic products did not accumulate. Thus, it is concluded that *T. roseopersicina* was growing phototrophically and did not use its chemolithotrophic potential at any time. It has been reported that *Chromatium* is able to photorespire in the presence of oxygen, resulting in the production of glycolate [22]. At present no information is available for *T. roseopersicina*, except that in the experiments reported here, no organic products accumulated in the culture medium. However, it is possible that glycolate or related products may have been produced and re-assimilated and that photorespiration and photoheterotrophy occurred simultaneously.

It has been demonstrated that photolithotrophically growing cells of *T. roseopersicina* when shifted to oxic conditions preferred to photosynthesize rather than respire [2]. The cells gradually shifted to respiration and only after prolonged cultivation in the presence of oxygen, when photosynthesis became insufficient because of low BChla contents [2]. The sustained phototrophic growth under shifting conditions is directly related to the ability to maintain the BChla content at a certain level. This was achieved by (1) an immediate start of BChla synthesis when the conditions became anoxic, and (2) an increased rate of BChla synthesis ($V_c$) during the shorter anoxic periods. In comparison with the culture grown under continuously anoxic conditions, a 48% higher rate of BChla synthesis was observed during the anoxic periods of the regimen 16 h/8 h and 21 h/3 h (oxic/anoxic) (Table 1). Nevertheless, the specific BChla content of these cultures was lower than in continuously anoxic cultures, but was still sufficiently high to allow for the rate of phototrophic growth rate imposed on the culture. This indicates that the cultures grown under regimens with oxic periods of 12 h or less contained 5–8 times more BChla than necessary for the growth rate imposed.

The present experiments were performed at a dilution rate of 0.03 h⁻¹ and in a 24-h cycle the average specific growth rate equaled the dilution rate. Attention is drawn to the fact that the specific BChla content depends not only on the length of the oxic period (see Table 1), but also on the dilution rate and light intensity. A mathematical description of the fluctuations observed under oxic/anoxic regimens allows a prediction of the impact of the dilution rate. The dynamics of the BChla concentrations in *T. roseopersicina* cultures can be calculated using the formula:

$$\frac{d[BChla]}{dt} = -D \cdot [BChla] + V_c \cdot x \quad (eq. 1)$$

in which $t$ represents time (h), $D$ the dilution rate (h⁻¹), $V_c$ the specific rate of BChla synthesis ($\mu\text{g} \cdot \text{mg}^{-1} \text{protein} \cdot \text{h}^{-1}$), and $x$ the protein concentration (mg·l⁻¹). The analytical solution of this equation is

$$[BChla]_t = \frac{V_c \cdot x}{D} + K \cdot e^{-D \cdot t} \quad (eq. 2)$$

in which $K$ represents the integration constant, which is dependent on the boundary conditions.

During the oxic periods BChla synthesis did not occur ($V_c = 0$), but the time course of BChla showed a time-dependent equilibrium coinciding with the total length of a 24-h cycle, thus:

$$[BChla]_t = [BChla]_{t-24} \quad (eq. 3)$$

In cultures subjected to regimens of 12 h/12 h, 16 h/8 h, 21 h/3 h (oxic/anoxic) it was observed that the specific BChla synthesis rate $V_c$ during the entire anoxic periods was maintained at 1.2 $\mu\text{g} \cdot \text{mg}^{-1} \text{protein} \cdot \text{h}^{-1}$. In all regimens, $V_c$ was zero during the oxic periods and protein showed little variation throughout. Assuming that the protein concentration ($x$) was constant, the limits of the specific BChla content are described by equa-
Fig. 5. Specific BChla content of *T. roseopersicina* strain M1 in continuous culture (*D* = 0.03 h⁻¹) subjected to various oxic/anoxic regimens. The symbols (●) indicate the highest and the lowest BChla content at the end of the anoxic and oxic periods, respectively. The theoretically predicted BChla content is indicated by the lines, in which A and B represent the situation at the end of the anoxic and oxic period, respectively. It is assumed that the fluctuations are bound by a maximum BChla content (27 μg·mg⁻¹ protein: dashed line) or a maximum BChla synthesis rate during the anoxic periods (1.2 μg·mg⁻¹ protein·h⁻¹: dotted line).

Equations 4 and 5:

\[ A = \frac{V_c}{D} \cdot \frac{1-e^{-D \cdot (24-I)}}{1-e^{-24 \cdot D}} \]  
\[ B = A \cdot e^{-D \cdot l} \]

in which *A* represents the predicted BChla content at the end of the anoxic period (i.e. the highest value), *B* the predicted BChla content at the end of the oxic period (i.e. the lowest value), and *I* the length (in h) of the oxic period in a 24-h cycle.

The fluctuations of the BChla content would thus be completely determined by the specific BChla synthesis rate (*V_c*) and the dilution rate (*D*). It was observed however, that *V_c* was not independent of the regimen employed. When cultivated at regimens with oxic periods of 7.5 h and shorter the average rate must have been lower than 1.2 μg·mg⁻¹ protein·h⁻¹, because otherwise the specific BChla content at the end of the anoxic periods (*A*) would have been higher than observed (Fig. 5: compare with dotted line). However, at light saturation, *T. roseopersicina* contained maximally 27 μg BChla·mg⁻¹ protein (Table 1).

In regimens with oxic periods of 12 h and longer, the specific BChla content was always lower than the maximal value of 27 μg·mg⁻¹ protein (Table 1 and Fig. 5: compare with dashed line). Under these conditions BChla synthesis during anoxic periods occurred at a constant rate of 1.2 μg·mg⁻¹ protein·h⁻¹ which is apparently the maximal value of *V_c*. Consequently, the specific BChla content decreased with increasing length of the oxic period (Eq. 4, 5 and Fig. 5). Obviously, under these conditions the anoxic periods were not long enough to allow the organism to reach the maximal BChla content.

The fluctuations of the specific BChla content are thus determined by two characteristic features, namely the maximal specific BChla content (27 μg·mg⁻¹ protein) and the maximal rate of BChla synthesis (1.2 μg BChla·mg⁻¹ protein·h⁻¹). The observed and the predicted values are shown in Fig. 5. When cultivated at regimens with oxic periods shorter than 10.5 h the average value of *V_c* was lower than the maximal value. At present, it is not known whether BChla is synthesized at a constant submaximal rate in the anoxic period, or whether it starts at maximal rate and subsequently slows down.

On the basis of the mathematical description given, the impact of the average specific growth rate (\( \mu \), being equal to the dilution rate *D*) on the specific BChla content of *T. roseopersicina* growing at various aeration can be predicted. At saturating light intensities, growth at lower \( \mu \) values will result in the maximal BChla at much longer oxic periods. In other words, the decrease of the specific BChla content during long oxid periods, due to a cessation of pigment synthesis in combination with continued growth, can be fully compensated for during short anoxic periods. This phenomenon is further enhanced by the fact that the cells can reach the same average specific growth rate at 1/5 to 1/8 of their maximal BChla content.

It is concluded that *T. roseopersicina* strain M1 is able to adapt very well to oxic/anoxic cycles by maintaining phototrophic growth and consequently high cell yields.
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