Chemolithotrophic growth of the phototrophic sulfur bacterium
Thiocapsa roseopersicina

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1. SUMMARY

Chemotrophic growth capacities of the purple sulfur bacterium Thiocapsa roseopersicina strain M1 were studied in continuous culture under thiosulfate limitation.

Pigment synthesis was completely inhibited upon a shift from anaerobic to semi-aerobic conditions (52 μM O₂) in the light, but no active breakdown occurred. During the transient state, the cells grew in a mixed photo- and chemolithotrophic mode; the specific respiration rate gradually increased with a concomitant drop in the bacteriochlorophyll a content. Photolithotrophically grown cells have the ability to respire. It was concluded that photosynthesis and respiration compete for electrons, but that photosynthesis is preferred under electron donor-limiting conditions, when the cells still contain large amounts of pigments. Eventually, a fully chemolithotrophic steady state was attained.

The chemolithotrophic growth of T. roseopersicina was studied in the dark under semiaerobic conditions at various dilution rates. The maximum specific growth rate was 68% of the maximum attainable growth rate under photolithotrophic conditions. The growth affinity for thiosulfate was high (Kₘ = 1.5 μM). The yield on thiosulfate under chemolithotrophic conditions exceeded that of thiobacilli. Oxygen uptake was studied in short-term experiments. It was shown that respiration in T. roseopersicina has a Kₘ of approx. 1 μM O₂.

The ecological importance for T. roseopersicina of chemolithotrophic growth and pigment content is discussed with respect to the occurrence of T. roseopersicina in laminated microbial ecosystems and its possible competition with colorless sulfur bacteria.

2. INTRODUCTION

The purple sulfur bacterium T. roseopersicina is commonly found as one of the dominant anoxygenic phototrophic bacteria in laminated microbial ecosystems developing in the surface layers of sediments (microbial mats) [1,2]. These microbial mats occur in hypersaline [3] and marine environments [4], and consist of a green top layer containing cyanobacteria, and often of a layer with purple sulfur bacteria just underneath. The environmental conditions in the mat are characterized by...
steep gradients of oxygen and sulfide, which shift strongly during the day-night cycle [5]. During the night, the cyanobacteria are confronted with sulfide. However, in the daytime their activities result in elevated concentrations of oxygen, and the layer of purple sulfur bacteria underneath is confronted with semi-aerobic conditions. In microbial mats, the non-motile _T. roseopersicina_ is exposed to oxygen during the day [4], and anaerobic conditions occur at night [4,5]. It is therefore of great ecological interest to know the reactivity of _T. roseopersicina_ under these conditions.

_T. roseopersicina_ is not a strict anaerobe. This purple sulfur bacterium not only maintains its viability in the presence of oxygen, but can even grow in a chemolithotrophic mode with sulfide or thiosulfate as electron donor [6]. The capacity for chemolithotrophic growth is quite common among members of the Chromatiaceae, as demonstrated for _Amoebobacter roseus_ [7], _Chromatium vinosum_, _Chromatium minus_, _Chromatium violascens_, _Chromatium gracile_ and _Thiocystis violacea_ [8]. For _T. roseopersicina_ strain BBS, it was shown that ribulose bisphosphate carboxylase (RuBisCo) was present and active in aerobically cultivated cells [9]. Thus, CO₂ is assimilated using the reductive pentose phosphate cycle, and the chemolithotrophic growth of purple sulfur bacteria is therefore comparable to that of thiobacilli.

Besides chemolithotrophic growth, some purple sulfur bacteria can grow chemo-organoheterotrophically [10], as has been shown for _Ectothiorhodospira shaposhnikovii_ with acetate as substrate [11].

In purple non-sulfur bacteria [12] and purple sulfur bacteria [13], oxygen represses bacteriochlorophyll and carotenoid synthesis, and thereby inhibiting photolithotrophic growth. There is probably a relation between pO₂ and the pigment content in purple sulfur bacteria [9].

The consumption of thiosulfate and sulfide by _T. roseopersicina_ under aerobic conditions in the dark is coupled to the reduction of O₂ [6,9]. Aerobic respiration also has been demonstrated in some purple sulfur bacteria cultivated photolithotrophically under anaerobic conditions as was shown as early as 1941 by Van Niel [14]. Endogenous respiration has been observed in several species, but respiration is normally strongly stimulated after the addition of sulfide or thiosulfate [9]. The maximum oxygen uptake rate is dependent on the culture conditions, being greater for chemolithotrophically than for photolithotrophically cultivated cells [9]. Nothing is known about the _Kₘ_ for oxygen of the respiratory system of purple sulfur bacteria.

The aim of the present study was to investigate the impact of oxygen in ecologically relevant concentrations on the growth performance and growth mode of _T. roseopersicina_.

### 3. MATERIALS AND METHODS

#### 3.1. Bacterial strain

All experiments were carried out with _T. roseopersicina_ M1, isolated from a laminated microbial ecosystem on the island of Mellum (F.R.G.).

#### 3.2. Medium and growth conditions

The organism was cultivated in ASN-III medium [15] with the following modifications. Nitrogen was supplied as NH₄Cl (0.2 g l⁻¹), and nickel as NiCl₂ · 6H₂O (0.019 mg l⁻¹). Vitamin B₁₂ was added (0.02 mg l⁻¹), and citrate omitted. Carbonate served both as carbon source and buffer, and was added to a final concentration of 2 g l⁻¹. These modifications were made so that one medium would be capable of supporting the growth of both cyanobacteria and purple sulfur bacteria.

#### 3.3. Procedure

The organism was grown in continuous culture as described by Beeftink and van Gemerden [16]. Thiosulfate served as electron donor and was the limiting substrate. Experiments in the light were carried out at intensity of 110 μmol m⁻² s⁻¹ (incandescent light). The dilution rate (D) was maintained as described for the separate experiments. The temperature was 25°C and the pH was kept at 8.0 with the aid of a pH-stat.

During cultivation under semi-aerobic conditions, the oxygen concentration was continuously monitored with a Yellow-Springs oxygen electrode.
connected to a custom-made polarographic measuring and controlling device. The oxygen concentration was kept constant at 25 ± 4% of air saturation, which corresponds to 52 ± 8 µM at the salinity and temperature conditions employed. This was performed by a controlled pulsing of air in the culture (0.1 bar pressure at a flow rate of 10 1·h⁻¹). The specific oxygen uptake rate \( q_{O_2} \) was determined in the culture from the linear decrease of the oxygen concentration after such pulses of oxygen.

The maximum \( O_2 \)-uptake rate \( (V_{\text{max}}) \), the endogenous \( O_2 \)-uptake rate, and the saturation constant \( (K_m) \) for oxygen were determined on subsamples in a separate vessel. The polarograph was connected via an analog-digital converter to an Apple IIe computer. Readings of the oxygen concentration were taken automatically at 30-s intervals (endogenous respiration) or 3-s intervals (maximum \( O_2 \)-uptake rates and \( K_m \)-determinations) and stored on disk. The maximum \( O_2 \)-uptake rate was determined after the addition of thiosulfate to a final concentration of 500 µM. Since thiosulfate does not react abiotically with oxygen all rates were due to biotic processes.

The maximal oxygen uptake rate \( (V_{\text{max}}) \) was calculated from the linear part of the oxygen depletion curve. The \( K_m \) was calculated from the non-linear part of this curve using its mathematical description:

\[
V_{\text{max}} \cdot t = S_0 - S_t + K_m \cdot \ln(S_0/S_t)
\]

in which \( S_0 \) and \( S_t \) are the concentrations of oxygen at times zero and \( t \), respectively [17].

Thiosulfate was determined colorimetrically [18]. Elemental sulfur was determined spectrophotometrically in methanol extracts of whole cells [19]. Bacteriochlorophyll \( a \) (BChla) was determined spectrophotometrically in methanol extracts of whole cells using an extinction coefficient of 84.4 g⁻¹·1·cm at 772 nm. Protein was determined with the Folin phenol reagent [20] after extraction of elemental sulfur and BChla with methanol and subsequent solubilization of the pellet in 1 N NaOH at 100°C. Total sugar was assayed with the anthrone reagent [21] with glucose as a standard. The anthrone method essentially determines C-6 sugars.

3.4. Redox calculations

In order to enable calculation of the redox balance, all electron-consuming and electron-donating reactions were normalized to specific rates using protein as a relative measure of cell material. This permits the direct comparison of all specific rates. Electron-donating reactions have a negative sign and electron-consuming reactions a positive sign [22]. Protein concentrations were correlated with the quantity of electrons required for the synthesis of structural cell material using a conversion factor, \( f_p \), determined as being 0.300 mmol e⁻·(mg prot.)⁻¹. A conversion factor of 2.05 mmol e⁻·(mg Ncell)⁻¹ was reported previously for \( C. \ vinosum \) [22]; this is equivalent to 0.328 mmol e⁻·(mg prot.)⁻¹. Glycogen concentrations were correlated with the quantity of electrons required for its synthesis using a conversion factor \( f_g \), being 0.136 mmol e⁻·(mg glycogenmonomer)⁻¹ [22]. The specific rate of glycogen synthesis is designated as \( V_6 \) [22].

In \( T. \) roseopersicina, the oxidation of thiosulfate under aerobic and anaerobic conditions can be described by a sequence of reactions:

\( \text{S}_2\text{O}_3^{2-} + 2 \text{e}^- \rightarrow \text{S}^2^- + \text{SO}_3^{2-} \) \hspace{1cm} (2)

\( \text{S}^2^- \rightarrow \text{S}^0 + 2 \text{e}^- \) \hspace{1cm} (3)

or:

\( \text{S}_2\text{O}_3^{2-} \rightarrow \text{S}^0 + \text{SO}_3^{2-} \) \hspace{1cm} (4)

followed by:

\( \text{SO}_4^{2-} + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2 \text{e}^- + 2 \text{H}^+ \) \hspace{1cm} (5)

\( \text{S}^0 + 4 \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 6 \text{e}^- + 8 \text{H}^+ \) \hspace{1cm} (6)

Reaction (2) is catalyzed by the enzyme thiosulfate reductase. Reaction (4) is catalyzed by the enzyme rhodanase. The presence of both enzymes has been demonstrated in \( T. \) roseopersicina strain BBS [23]. In cultures of purple sulfur bacteria, the concentrations of sulfite and sulfide due to the oxidation of thiosulfate are extremely low. Consequently, the specific rates of Eqns. 2 and 4 (the cleavage of thiosulfate), the oxidation of sulfide to sulfur (Eqn. 3) and the oxidation of sulfite (Eqn. 5) are summarized and estimated as \( V_1 \), analogous to the oxidation of sulfide to elemental sulfur [22]. The specific rate of sulfur formation is numeri-
cally equivalent to the specific rate of thiosulfate cleavage. The specific rate of sulfur oxidation, designated as \( V_2 \), can thus be calculated from the rate of thiosulfate consumption and the rates of formation of elemental sulfur and protein.

During photolithotrophic growth, thiosulfate is used as electron donor for assimilatory purposes only. The result is the synthesis of biopolymers which balance the electron donating reactions. Under chemolithotrophic conditions, this is not to be expected because part of the substrate is respired. The imbalance between the amount of electrons donated and the amount of electrons consumed in the formation of cell-polymers is designated as the rate \( V_X \) and calculated according to \( V_1 + V_2 + \mu + V_6 + V_X = 0 \). This imbalance can be due to respiration and excretion (\( V_X = V_R + V_{exc} \)), but the excretion of organic compounds by \( T. \) roseopersicina is negligible, and thus \( V_R \) equals \( V_X \) in practice. Therefore, the specific rate of respiration (\( V_R \)) can be fairly accurately measured from this imbalance as described above. In the RESULTS section the value of the indirectly calculated \( V_R \) will be compared with the actual specific oxygen uptake rate.

In the calculations of the specific rates, corrections have been made for wash-out and for the inflow of thiosulfate from the reservoir bottle.

4. RESULTS

\( T. \) roseopersicina was grown anaerobically in the light with thiosulfate as the limiting factor. After the establishment of a steady state, the culture conditions were shifted to semi-aerobic (i.e., 25% air saturation), while the dilution rate and illumination intensity were kept constant. Eventually a new steady state was reached. Data on protein, total sugar, elemental sulfur, and BChl obtained in an experiment run at a dilution rate of 0.019 h\(^{-1}\) are shown in Fig. 1. During the entire experiment, the concentration of thiosulfate in the culture vessel was below 3 \( \mu \text{M} \). Immediately after the shift, the protein concentration declined, but stabilized after about 6.5 volume changes at approx. one-third of its original value (Fig. 1A). During the transient state, the concentration of elemental sulfur initially increased, but eventually returned to the original value. In relation to the decreased protein concentration, the sulfur content in the chemolithothrophic steady state was about 3 times that in the preceding photolithothrophic steady state. The time course of total sugar was very similar to that of elemental sulfur (Fig. 1A). The time-course of BChl \( a \) is shown in Fig. 1B. The light-harvesting pigments were washed out of the culture according to the theoretical wash-out curve. During the experiment, the color of the culture changed from pink to milky white. The BChl \( a \) concentration finally dropped below the limit of detection (10 \( \mu \text{g} \cdot \text{l}^{-1} \)). The organism was therefore growing fully chemolithotrophically at the end of the shift experiment.

In order to gain insight into the utilization of thiosulfate, and in particular into the growth mode during the transient state, calculations of the
specific rates of the electron-consuming and electron-donating reactions were made. The results of these calculations are shown in Fig. 2, in which \( qO_2 \) is also included.

During the phototrophic steady state, the sum of the specific rates of the electron-donating reactions (\( V_1 = -0.0056 \text{ h}^{-1}, V_2 = -0.0165 \text{ h}^{-1}, \) in total \(-0.0221 \text{ h}^{-1}\)) was balanced by growth and glycogen synthesis (0.019 h\(^{-1}\) and 0.0027 h\(^{-1}\), respectively, in total 0.0217 h\(^{-1}\)). The shift to semi-aerobic conditions resulted temporarily in a somewhat slower oxidation of sulfur, but both \( V_1 \) and \( V_2 \) gradually changed to the values corresponding to the chemolithotrophic steady state. Initially, the specific growth rate (\( \mu \)) decreased from 0.019 h\(^{-1}\) (steady state) to less than half this value. This was apparently not due to shortage of electrons, since the rate of glycogen synthesis increased simultaneously. After 75 h, \( \mu \) began to increase again, showed a slight overshoot at 100 h, and then stabilized at 0.019 h\(^{-1}\), the dilution rate. During the transient phase, the calculated specific rate of electrons being used for respiration (\( V_R \), see MATERIALS AND METHODS) showed a gradual increase with time from zero (photolithotrophic steady state) to 0.038 h\(^{-1}\) (chemolithotrophic steady state). The calculated rate, \( V_R \), showed a good correlation with the measured \( qO_2 \) \((r = 0.959, N = 11)\). The direct comparison between electrons respired and oxygen used is possible after the dimension of \( V_R \) has been changed using the conversion factor \( f_1 (0.300 \text{ mmol e}^- \cdot (\text{mg prot})^{-1}) \). This yields

\[
V_R' = V_R \cdot f_1 = 4.6 \cdot qO_2 - 2.2
\]

i.e., the respiration of 1 mol oxygen corresponds to 4.6 mol electrons delivered by \( V_R \).

During the transient state, samples were taken to estimate the endogenous respiration rate, and the maximum respiration rate (\( V_{\text{max}} \), both in the light and in the dark. Table 1 lists the results.

Attention is focused on the fact that phototrophically grown cells are able to respire. However, in the light, a strong reduction of the maximum attainable rate was observed. In the transient state, the maximum respiration rate initially declined, both in the light and in the dark, and the inhibitory effect of illumination remained. However, in the newly attained chemolithotrophic steady state, the maximum respiration rate exceeded by far the values in the preceding photolithotrophic steady state. After 2.7 and 3.2 volume changes, the maximum rate was reduced only marginally in the light, and in the chemolithotrophic steady state, no effect of light was observed at all.

The \( qO_2 \) directly estimated in the main culture

![Fig. 2. Time course of the specific electron-donating and electron-consuming rates determined in the experiment of Fig. 1. \( V_1 \), Specific rate of formation of elemental sulfur directly, and through sulfide oxidation (see MATERIALS AND METHODS) from thiosulfate; \( V_2 \), specific rate of sulfur oxidation; \( \mu \), specific growth rate; \( V_6 \), specific rate of glycogen formation; \( V_R \), calculated specific rate of respiration; \( qO_2 \), specific oxygen uptake rate, measured directly.](https://academic.oup.com/femsec/article-abstract/3/2/117/470508)
Table 1
Oxygen uptake rates and \( K_m \) values for oxygen uptake of \( T. \) roseopersicina strain M1

Samples were taken from the photolithotrophic steady state, the transient state, and the chemolithotrophic steady state. The maximum \( O_2 \)-uptake rate was determined after addition of thiosulfate to a final concentration of 500 \( \mu \)M. The rates are expressed as nmol \((\text{mg protein})^{-1} \cdot \text{min}^{-1}\), and \( K_m \) in \( \mu \)M \( O_2 \).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Endogenous</th>
<th>Maximum</th>
<th>( q_{O_2} )</th>
<th>( K_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light</td>
<td>dark</td>
<td>light</td>
<td>dark</td>
</tr>
<tr>
<td>Photolithotrophic steady state</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t = 72 )h</td>
<td>0</td>
<td>12.7</td>
<td>84.7 (57)</td>
<td>149.7 (100)</td>
</tr>
<tr>
<td>( t = 144 )h</td>
<td>0</td>
<td>16.5</td>
<td>36.4 (51)</td>
<td>71.9 (100)</td>
</tr>
<tr>
<td>( t = 169 )h</td>
<td>8.7</td>
<td>19.8</td>
<td>248.7 (94)</td>
<td>266.1 (100)</td>
</tr>
<tr>
<td>Chemolithotrophic steady state</td>
<td>5.6</td>
<td>13.5</td>
<td>205.7 (96)</td>
<td>215.4 (100)</td>
</tr>
</tbody>
</table>

(Fig. 2) was in all cases much lower than the \( V_{max} \) estimated in the subsamples. Immediately after the shift to semiaerobic conditions, the actual \( q_{O_2} \) was only 2% of \( V_{max} \) while in the chemolithotrophic steady state, a 7.6-fold overcapacity was observed.

Because the cells in the culture are continuously supplied with fresh medium containing thiosulfate, the \( q_{O_2} \) is always higher than the endogenous rate estimated in subsamples under starvation conditions. The endogenous rate was drastically reduced in the light compared to the dark (Table 1). In the photolithotrophic steady state, the endogenous rate in the light was zero. The \( K_m \) for oxygen uptake (with thiosulfate added) was calculated from the oxygen-depletion curve. The \( K_m \) did not differ in the light or in the dark, and was estimated as 1 \( \mu \)M \( O_2 \).

Finally, the chemolithotrophic growth of \( T. \) roseopersicina in the dark in the presence of oxygen (52 \( \mu \)M) was studied. The steady-state contents of protein, elemental sulfur and total sugar are shown in Fig. 3. For each steady state, redox calculations were performed. The results for dilution rates of 0.019 \( h^{-1} \), 0.027 \( h^{-1} \), 0.038 \( h^{-1} \), and 0.045 \( h^{-1} \) are shown in Table 2. The results show that about 70% of the substrate is respired to provide energy for the fixation of \( CO_2 \) and the subsequent synthesis of cell material.

Using the direct linear plot [24], the \( K_m \) for thiosulfate was estimated as 1.5 \( \mu \)M, and the \( \mu_{max} \) 0.052 \( h^{-1} \). These values have been used to calculate the curve relating the specific growth rate and the concentration of thiosulfate shown in Fig. 4. For comparison, under photolithotrophic condi-

![Fig. 3. Steady-state data on protein, total sugar and sulfur for chemolithotrophically growing \( T. \) roseopersicina strain M1 at various dilution rates (\( D \)) in the dark on thiosulfate under semi-aerobic conditions (52 \( \mu \)M \( O_2 \)). The curves are extrapolated to \( D = 0.052 \) h\(^{-1}\), which equals \( \mu_{max} \) as calculated using the data of Fig. 4.](https://academic.oup.com/femsec/article-abstract/3/2/117/470508)
Table 2
Redox calculations performed on steady-state data of chemolithotrophically grown *T. roseopersicina* strain M1 cultivated in the dark at 52 \( \mu M \) \( O_2 \)

The quantities are expressed as mmol electrons l\(^{-1}\)

<table>
<thead>
<tr>
<th>Dilution rate ( (h^{-1}) )</th>
<th>0.019</th>
<th>0.027</th>
<th>0.038</th>
<th>0.045</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Input ( (S_R, 8) )</td>
<td>33.56</td>
<td>35.01</td>
<td>31.96</td>
<td>32.36</td>
</tr>
<tr>
<td>B Residual thiosulfate ( (s.8) )</td>
<td>&lt; 0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>C Sulfur ( (S^0, 6) )</td>
<td>0.47</td>
<td>1.43</td>
<td>2.48</td>
<td>6.06</td>
</tr>
<tr>
<td>D Utilized ( (A - B - C) )</td>
<td>33.09</td>
<td>33.57</td>
<td>29.44</td>
<td>26.22</td>
</tr>
<tr>
<td>E Structural cell material</td>
<td>7.41</td>
<td>7.78</td>
<td>6.73</td>
<td>5.26</td>
</tr>
<tr>
<td>F Sugar</td>
<td>3.04</td>
<td>2.50</td>
<td>1.75</td>
<td>1.05</td>
</tr>
<tr>
<td>G Total produced ( (E + F) )</td>
<td>10.45</td>
<td>10.28</td>
<td>8.47</td>
<td>6.30</td>
</tr>
<tr>
<td>H Synthesis ( (% ) of total ( G/D )</td>
<td>32</td>
<td>31</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>J Respiration ( (% ) of total ( G/D )</td>
<td>68</td>
<td>69</td>
<td>71</td>
<td>76</td>
</tr>
</tbody>
</table>

Fig. 4. Steady-state data on the residual concentration of thiosulfate \( (s) \) and the specific growth rate as determined from Fig. 3. The solid line is the curve calculated according to Monod kinetics, using the direct linear plot [24].

5. DISCUSSION

The ability of *T. roseopersicina* strain BBS to grow as a chemolithotroph with thiosulfate as electron donor has been demonstrated by Bogorov [6]. This study shows that *T. roseopersicina* strain M1 has the potential to grow remarkably well as a chemolithotroph, in relation to its photolithotrophic growth capacities.

When a photolithotrophically grown culture of *T. roseopersicina* strain M1 was shifted to semi-aerobic conditions, the carotenoids and BCHla were washed out according to the theoretical wash-out curve. This indicates a complete cessation of pigment synthesis in the presence of oxygen, but no active breakdown. The repression of BCHla synthesis by oxygen has already been shown by Cohen-Bazire et al. [12] for purple nonsulfur bacteria, and by Hurlbert [13] for purple sulfur bacteria. In our experiments, the pigments were almost completely lost after 5 volume changes, and the cells grew in a chemolithotrophic mode. This was confirmed by a high in situ \( qO_2 \) and a strong discrepancy between thiosulfate oxidation and the formation of cell material.

During the whole experiment there was a good correlation between the specific respiration rate \( V_R \) and the specific \( qO_2 \). From this correlation a ratio of 1 mol \( O_2 \) consumed to 4.6 mol of electrons delivered was determined. This ratio is close to the expected stoichiometry, according to Eqn. 7.

\[
O_2 + 4 e^- + 2H_2O \rightarrow 4 OH^- \quad (7)
\]

This confirms that excretion in this species is of minor importance.

The observation that *T. roseopersicina* upon shifting to semi-aerobic conditions initially continued to grow in a phototrophic mode \( (qO_2 \) in the light only 2\% of its potential) would appear to contradict the finding that these cells possess the ability to perform respiration. The latter capacity
seems to be constitutive in this species. It was found that the uptake of oxygen was repressed in the light. This indicates that the electron transport chains for respiration and photosynthesis are connected to each other, e.g., by common redox couples. Conceivably, photosynthesis and respiration have to compete for electrons. When the supply of electrons is rate-limiting, more severe competition between photosynthesis and respiration will occur than in the situation with added thiosulfate. The very low $qO_2$ and the complete cessation of endogenous respiration in the light indicates that photosynthesis is preferred under electron donor-limiting conditions. With reduced BCHla levels, photosynthesis becomes less efficient and the culture starts to respire. When the pigments are completely washed out, competition is no longer feasible, and the $qO_2$ increases further. The idea that there is competition for electrons between photosynthesis and respiration may help to explain the phenomena observed in the transient state.

Repression of respiration in the light could be due to feedback control by a high $\Delta \psi$ or to competition at the level of the electron transport chain [25]. When the supply of electrons is rate-limiting, competition at electron transport chain level is more probable.

At 25% air saturation (52 $\mu$M oxygen), pigment synthesis is completely repressed and the cells finally become devoid of light-harvesting pigments. In nature, it would be a disadvantage for this organism to lose its photolithotrophic growth capacity completely. For T. roseopersicina and Thiocystis violacea, it has been shown that at low, but unknown, concentrations of oxygen, the cells still contained low levels of BCHla and carotenoids [6,8]. Kämpf and Pfennig [26] measured for C. vinosum D an inverse relation between oxygen concentration and BCHla-content. When grown at 30 $\mu$M O$_2$, this species contains 2 $\mu$g BCHla $\cdot$ (mg prot)$^{-1}$, while at 8 $\mu$M O$_2$ it contains 10 $\mu$g BCHla $\cdot$ (mg prot)$^{-1}$. It is tentatively assumed that the same type of relation holds for T. roseopersicina strain M1.

T. roseopersicina strain M1 has a very low $K_m$ for oxygen uptake under photolithotrophic, chemolithotrophic, and transient conditions. This may be ecologically important, because it means that T. roseopersicina can compete efficiently for limiting oxygen in the dark. It also means that a T. roseopersicina population has the possibility of scavenging O$_2$ from the environment. The resulting anaerobic conditions stimulate pigment synthesis. On the other hand, if the electron donor supply is rate-limiting, then pigment-containing cells will show much reduced respiration in the light. The impact of lower illumination is not known at present.

The growth performance of T. roseopersicina in the dark with 52 $\mu$M O$_2$ is noticeably good. The maximal attainable specific growth rate is 68% of that measured under phototrophic conditions, and the affinity for both oxygen and thiosulfate is very high. This illustrates the remarkable flexibility of this species. Transmission electron micrographs of T. roseopersicina strain BBS cultivated under aerobic conditions in the dark clearly show the presence of polysaccharide granules [9]. We observed that the content of hexose in T. roseopersicina strain M1 is higher under chemolithotrophic conditions than under photolithotrophic conditions (1 and 0.37 mg hexose $\cdot$ (mg prot)$^{-1}$, respectively). This effect is more pronounced at lower dilution rates.

Redox calculations showed that in chemolithotrophic cultures, 24–32% of all the electrons utilized resulted in the synthesis of cell material. For thiobacilli, values of 13–29% have been reported [27]. T. roseopersicina attains a relatively high yield on thiosulfate, compared to thiobacilli. In view of the fact that the lowest dilution rate tested was 37% of $\mu_{\text{max}}$, no conclusions can be drawn with respect to the importance of maintenance energy requirements.

Under chemolithotrophic conditions, T. roseopersicina has to compete with colorless sulfur bacteria. For thiobacilli, many relevant data have been gathered by cultivation in continuous culture [27]. The kinetic parameters of the different species are of interest with respect to the predicted outcome of competition between T. roseopersicina and thiobacilli. T. roseopersicina has a $K_s$ value of 1.5 $\mu$M and a $\mu_{\text{max}}$ of 0.052 h$^{-1}$. However, concentrations of thiosulfate in Thiobacillus sp. cultures are generally below the limit of detection.
and the maximum growth rates can be 7-8-fold higher [27,28]. Therefore growth affinity, defined as the initial slope of the μ–s curve (μ_max, K_m⁻¹) appears to be much higher for Thiobacillus sp., and it is expected that Thiobacillus sp. would outcompete T. roseopersicina in the continuous presence of oxygen with thiosulfate as a limiting substrate. However, aerobic/anaerobic transitions in the light can be expected to favor the photo-trophs.

With respect to Beggiatoa and related organisms, no information is available on the affinities for reduced sulfur compounds. This is due to problems encountered with chemostat cultures of these organisms. From growth curves determined in agar gradient tubes [29], it can be calculated that the maximum growth rate will be of the same order of magnitude as that determined for T. roseopersicina. Environmental factors of crucial importance include not only reduced forms of sulfur and oxygen, but also the intensity of the light. Jørgensen et al. [3] studied sulfur bacteria under Microcoleus chthonoplastes mats in hypersaline ponds at the oxygen-sulfide interface. The presence of a Beggiatoa population was correlated with the absence of light in the region 700–900 nm, whereas a Chromatium bloom was correlated with the presence of this light at this interface. T. roseopersicina coexists with cyanobacteria such as M. chthonoplastes in microbial mats. The fact that the sulfur bacterium is an efficient chemolithotroph is ecologically relevant, since M. chthonoplastes oxidizes sulfide to thiosulfate only, which can be used by T. roseopersicina [30]. Thiosulfate will also be formed by abiotic oxidation of sulfide, and will remain stable in the presence of oxygen [31].

Attention is drawn to the fact that T. roseopersicina cells growing completely chemolithotrophically are not detected by pigment analysis. The population may therefore occur closer to the surface of the sediment than is suggested by pigment profiles. However, more information is needed about the growth of T. roseopersicina at higher oxygen concentrations. Maximum growth rates of C. vinosum D decreased from 0.045 h⁻¹ at 40 μM O_2 to 0.015 h⁻¹ at 300 μM O_2 [26]. In the M. chthonoplastes layer, concentrations of oxygen far above air saturation, occur at higher light intensities.

Conceivably, T. roseopersicina performs best in situations where it can express both its photolithotrophic and chemolithotrophic capacities. This may be under microaerobic (0–20 μM O_2) conditions. However, stable conditions are rare in phototrophic communities, which are characterized by light–dark-induced fluctuations. More research is needed into the growth and pigment kinetics of T. roseopersicina under semi-aerobic-anaerobic regimes (a pulsed oxygen supply). Such studies will throw more light on the ecological niche of T. roseopersicina in sediment ecosystems.

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