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# Comparative anoxygenic photosynthetic capacity in 7 strains of a thermophilic cyanobacterium

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**Abstract.** The capacity for anoxygenic photosynthesis and other physiological traits related to sulfide tolerance were compared in several strains of the thermophilic cyanobacterium *Oscillatoria amphigranulata.* Strains were isolated from hot springs in which the environmental sulfide over *O. amphigranulata* microbial mats spanned a range from 0.2 to 1 mM. Great differences in the capacity for anoxygenic photosynthesis existed among the isolates but these correlated in a predictable manner with the sulfide content of the springs. The time required for commencement of anoxygenic photosynthesis and the degree of initial sensitivity of Photosystem II to sulfide did not correlate with environmental sulfide levels. Kinetic parameters of sulfide consumption indicate uniformly low affinities for sulfide ($K_m$ of about 1 mM) but differences among strains in $V_{max}$.

**Key words:** Cyanobacteria – Anoxygenic photosynthesis – Sulfide tolerance – Thermophiles – Microbial mats – Hot springs – *Oscillatoria amphigranulata*

Sulfide has been shown to potently inhibit Photosystem II (PSII) in some cyanobacteria and *Nicotiana* chloroplasts (Oren et al. 1979) and essentially all organisms are poisoned at high concentration. Nevertheless, cyanobacteria are known to inhabit high sulfide environments, although the number of species is very restricted. Most of the cyanobacteria of sulfureta have mechanisms of coping with high sulfide concentrations that include facultative anoxygenic photosynthesis, increased tolerance of PSII to sulfide, or both (see Cohen et al. 1986).

*Oscillatoria* cf. *amphigranulata* is a thermophilic cyanobacterium characteristic of the sulfide-containing hot springs of New Zealand up to a temperature of 56°C (Castenholz 1976). Simultaneous anoxygenic and oxygenic photosynthesis under sulfide was demonstrated in a strain of this species (Castenholz and Utkilen 1984a). The presence of sulfide is positively correlated with the occurrence of *O. amphigranulata* in hot springs, although the range of concentrations at which it has been found is from 0.05 to over 2 mM (Castenholz 1976; this work). A survey was undertaken to assess the constancy of the sulfide tolerant physiology among disjunct natural subpopulations of *O. amphigranulata*.

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*Abbreviations:* CAM, Chloramphenicol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PC, phycocyanin; PE, phycoerythrin; PSII, photosystem II

## Materials and methods

A total of 32 clones of thermophilic, filamentous cyanobacteria were isolated from samples obtained from 40–50°C hot-springs of the central volcanic region of the North Island, New Zealand. The isolations were carried out as described by Castenholz and Utkilen (1984a), from single trichomes gliding centrifugally from a central inoculum on agar plates. The isolates were all assigned to the species *Oscillatoria amphigranulata* van Goor on the basis of morphology. Six of the clones were rendered axenic and these were the main organisms in this study (see Table 1). Strains were either green or brown in color with c-phycoerythrin in the brown strains. All new strains are kept in the Culture Collection of Thermophilic Cyanobacteria at the University of Oregon. Their collection denominations are NZ-Source Number-Oa-clone number, e.g. NZ-13-Oa-2, but for the purpose of this paper they will simply be addressed by Source Number-Clone Number, e.g. 13-2.

During isolation, maintenance and through the experiments, DGN medium (Castenholz 1981) or DTN (with "Tricine" replacing glycylglycine as buffer in the same concentration) were used as a defined medium containing reduced nitrogen compounds. All the strains tested showed no or very poor growth when nitrate was the only source of nitrogen. The pH of the media was set at 8.0. Stock cultures were kept as described previously (Castenholz and Utkilen 1984a). Culturing at a larger scale for experimentation could not be performed in bubbler vessels in the fashion previously reported (Castenholz and Utkilen 1984a) since the filaments of the present strains had a strong tendency to clump together and attach to the vessel walls. Nevertheless, this tendency could be overcome by growing the cells under continuous, vigorous stirring, provided that this treatment was started during the early phases of culture growth. The experimental batch cultures were grown in sterile 1 l bottles stoppered with cotton plugs and containing a 5 cm magnetic bar.

Cultures were placed in a heated aquarium. The temperature was maintained with a Haake E12 submersible heater-circulator. Cultures were initially fed with 100 µl of a 1 M solution of $NaHCO_3$ prepared from separately sterilized water and powder. This addition raised the pH of the medium less than 0.05 units. Further additions were made along the growth period, taking care that pH did not rise over 8.1. Exponentially growing cultures were transferred by sterile means to the final experimental apparatus.

All experiments were carried out at a temperature of about 40°C and light intensity of 3.8 klx (67 µE $m^{-2}$ $s^{-1}$) produced by four four-foot cool white fluorescence tubes with 2.2 Amp ballasts. This light intensity corresponds to near-saturating light for both green and brown type *O. amphigranulata* (Mitchell 1979). Chlorophyll *a* was measured spectrophotometrically at 665 nm using $MgCO_3$-saturated methanol extracts after filter-clarification. A specific absorption coefficient (α) (1 cm) = 0.075 ml/g was used (Lenz and Zeitzschel 1968). Protein was assessed by the method of Smith et al. (1985) using a commercial kit (Sigma) after extraction of the protein in 1 M NaOH at 100°C for 20 min. Soluble sulfide was quantified by the method of Cline (1969). Thiosulfate was quantified according to Urban (1961). Sulfide stock solutions were prepared as described by Castenholz and Utkilen (1984a). Ultrapure DCMU was used in aqueous solution from a stock of $1 \times 10^{-4}$ M and was a gift from E.I. Dupont de Nemours, Wilmington, DE, USA. Chloramphenicol stock was prepared as an aqueous solution of 500 mg $l^{-1}$.

For the [$^{14}$C]-$HCO_3^-$ photoincorporation experiments, two main treatments were performed with clones that had never been in contact with sulfide since field sampling. Cultures were divided into two batches, one of which was supplied with sulfide (0.6 mM final concentration) and allowed to stand in the light at growth temperatures for a period of 3 h for physiological adaptation. After this, remaining sulfide was removed to undetectable levels with 5 min of sparging with a sterile gas mixture (1% $CO_2$ in 99% $N_2$). The second batch was used immediately, after sparging with the sterile gas mixture. The set of conditions for incubation of both batches was similar to that used for *O. amphigranulata* strains in the past (Castenholz and Utkilen 1984a). Conditions included: (1) an oxygenic photosynthesis control in the light with no additions, (2) a darkened control, (3) incubation in the light with sulfide added to reach 0.6 mM final concentration, (4) a darkened control for (3), (5) incubation with 0.6 mM sulfide and 7 µM DCMU in the light, (6) a dark control for (5), (7) incubation in the light with 7 µM DCMU and no sulfide, (8) incubation in the light with 0.6 mM sulfide, 7 µM DCMU and 50 mg $l^{-1}$ chloramphenicol and (9) incubation in the light with 0.6 mM sodium thiosulfate and 7 µM DCMU added. Sampling was done by syringe at regular time intervals. During incubation, vials were occasionally gently hand-shaken to prevent clumping of the trichomes. The suspension thus remained homogeneous for several hours. Sulfide usually needed to be brought back to initial levels only once during the incubations. The maximum variation was 0.3 mM. Fixed samples were filtered using 0.45 µm pore diameter cellulose triacetate membrane filters (Gelman Sciences), and possible carbonate precipitates removed by washing with a solution of 2% HCl, then washed again with distilled water. Filters were dried overnight at room temperature and placed in 20 ml scintillation vials containing 7 ml of Amersham "Phase Combining System" (PCS) scintillation cocktail and counted in a Beckman LS6800 scintillation counter.

For the sulfide depletion experiments, exponentially growing cultures were subdivided into three batches and set in stirred bottles as described above. The cotton plugs were replaced by rubber stoppers with two syringe needles. DCMU (7 µM final concentration) was added to halt the biogenesis of oxygen. One of the cultures received 50 mg $l^{-1}$ chloramphenicol to prevent further synthesis of protein. Finally a third batch was darkened by aluminum foil wrapping. The vessels were bubbled with a constant mixture of 1% $CO_2$ and 99% $N_2$ for 0.5 h, then sealed with the rubber stoppers. A flow of the gas mixture through the syringe needles for 1–2 min removed oxygen that may have leaked in during sealing. Sulfide, as a 100 mM solution at pH 8.2 was introduced in the bottles to give the desired final concentration, and the sulfide level followed with time. Possible thiosulfate production was also monitored. Samples were taken by turning the bottles upside-down and sucking with a syringe. With the aid of a three way stopcock the entrance of air was prevented and negative pressure was relieved with the sterile gas mixture.

Sulfide depletion time courses in these strains seemed to be fairly well described by inverse saturation kinetics. Regression analysis was used to fit the data from the depletion progress series to a linearized form of the integrated Michaelis-Menten model adapted to inverse saturation kinetics as described by Robinson and Characklis (1984). From the three linearizations available, the following was used since it has been proven to give the best estimates:

$$t/(S_0 - S_t) = (K_m/V_{max}) \ln (S_0/S_t)/(S_0 - S_t) + 1/V_{max},$$

where t is time, $S_t$ stands for concentration of substrate (sulfide) at time t and $S_0$ is concentration of substrate (sulfide) at time = 0. $V_{max}$ is the maximum rate of substrate consumption (rate of consumption at substrate saturation). $K_m$ is the "saturation constant" or the concentration of substrate at which V (rate of substrate consumption) is 1/2 of $V_{max}$. Sulfide/time data pairs were taken into consideration only after adaptation was complete and the depletion was obvious, that is, starting at the second point after depletion commencement.

## Results

From the original 32 clones isolated, six were successfully rendered axenic and characterized beyond morphology (Table 1). A major characteristic of these strains is the presence or absence of c-phycoerythrin (PE) as a major phycobilin. Three of the strains studied (19-2, 14-1, 11-3) possessed PE under normal culture conditions (cool white fluorescent lamps), although PE complement was preferentially lost in all of these strains when grown at the upper or lower limits of their temperature range and most conspicuously at 50–55°C (data not presented). These strains readily recovered PE when returned to normal growth conditions (40–45°C). Two of the strains (13-2 and 23-2) lacked PE and were green. 21-3.1 is a PE-lacking spontaneous variant of 21-3 (PE-endowed) that appeared as a patch in liquid culture during the course of the work. 21-3.1 was isolated by recloning from 21-3. 21-3 was not used in the experiments since it was not freed of a contaminating flexibacterium. NZ-Concert-Oa-1-gr is also an earlier green variant (Mitchell 1979) of a PE-containing strain (NZ-Concert-Oa-1, Castenholz and Utkilen 1984a) that was used for comparison. All new strains were unable to grow in the absence of reduced nitrogen compounds or grew very poorly after a second transfer in ammonia-free, nitrate containing medium. Temperature ranges were similar with optima between 35 and 45°C (data not shown). Active clumping and attachment to solid surfaces was recalcitrant only in the new strains, possibly enhanced by the very thin sheaths they developed.

All strains tested carried out DCMU-insensitive, light dependent incorporation of [$^{14}$C]-$HCO_3^-$ in the presence of sulfide, but no thiosulfate supported incorporation was detected in any, either before or after adaptation to sulfide. No adaptation to thiosulfate was attempted. In all cases no photoincorporation, or little (less than 5%), occurred if chloramphenicol was added to the incubation

**Table 1.** Characteristics of the isolates

| | Strain | 19-2 | 13-2 | 23-2 | 14-1 | 11-3 | 21-3.1 |
|---|---|---|---|---|---|---|---|
| Source | Spring | Cirque | Cirque | Par-8 | TB-88 | Spte-2 | Tok-10 |
| | Mat color[a] | YG to B | YG to B | G | YG to B | dull G | dull G |
| | pH[b] | 8.52 | 8.52 | 6.7 | 9.1 | 8.9 | 5.15 |
| | Sulfide (mM)[b] | 0.02 | 0.04 | 0.09 | 0.73 | 0.43 | 0.02 |
| *Isolate* | | | | | | | |
| Sheath | | thin | thin | thin | thin | thin | thin |
| Cell | Breadth (μm) | 3 | 3 | 3 | 3 | 3 | 3 |
| | Length (μm) | 1−2 | 1−2 | 1−3 | 1−4 | 1−3 | 1−3 |
| Behavior | Gliding | + | + | + | + | + | + |
| | Clumping | ++ | + | + | ++ | ++ | + |
| | Attachment | + | ++ | ++ | + | + | ++ |
| *Phycobilins* | | | | | | | |
| | PC | + | + | + | + | + | + |
| | c-PE | + | − | − | + | + | − |
| | Loss of c-PE[c] | ± | na | na | + | + | na |
| *Anoxygenic photosynthesis* | | | | | | | |
| | with sulfide | + | + | + | + | + | + |
| | with thiosulfate | − | − | − | − | − | − |
| | to sulfur | + | + | + | + | + | + |
| | to thiosulfate | − | − | − | − | − | − |
| *Nitrogen requirements* | | | | | | | |
| | Growth with | | | | | | |
| | ammonia | + | + | + | + | + | + |
| | urea | − | + | + | + | + | + |
| | nitrate | − | poor | poor | − | − | poor |
| Death at pH ± 8.8 | | + | + | + | + | nt | nt |
| Temperature | Range | 30−50 | <30−50 | 30−50 | <30− <55 | <30−55 | 30− <50 |
| | Optimum | 45 | 35−45 | 45 | 45 | 35−45 | 45 |

[a] YG: yellow-green; G: green; B: brown
[b] In waters over *O. amphigranulata* biofilms
[c] At extremes of temperature range for growth

nt: not tested
na: not applicable

vials before adaptation to sulfide, but some took place if added after adaptation was complete (Figs. 1, 3).

A lapse of time was clearly required by some of the strains (14-1, 19-2) (Figs. 1, 2) to start incorporation in the presence of sulfide and for others (13-2, 23-2) incorporation appeared to take place immediately after sulfide was added (Figs. 3, 4). These results contradict the fact that for all strains, chloramphenicol totally prevented the adaptation to sulfide. Additional trials with these strains showed the same pattern (data not shown). The correlation found between the absence of a conspicuous adaptation period and the lack of c-phycoerythrin (23-2 and 13-2 were green and showed no adaptation period, whereas 14-1 and 19-2 were brown and showed adaptation period) called for the testing of PE-lacking NZ-Concert-Oa-1-gr. An adaptation period for anoxygenic photosynthesis to commence had been demonstrated for its PE-containing, "parent" strain NZ-Concert-Oa-1 (Castenholz and Utkilen 1984a). The results obtained with this green variant (Fig. 5), show about a 1 h period of adaptation to sulfide, thereby discarding the hypothesis of a necessary linkage between immediate response to sulfide and pigment complement.

In all strains, except for NZ-Concert-Oa-1-gr, the time courses of photoassimilation of adapted cells in the presence of sulfide alone showed a biphasic progress. In the first phase (1−2 h) the rates of photosynthesis in the presence of sulfide alone did not differ from those of the incubation with sulfide plus DCMU. This coincidence illustrates the probable poisoning by sulfide of PS II, in that there is no apparent contribution of oxygenic photosynthesis. The inhibitory effect of sulfide is even more conspicuous in the strains that show a lengthy adaptation period for anoxygenic photosynthesis (14-1, 19-2, NZ-Concert-Oa-1-gr) since there was no photoincorporation under sulfide before anoxygenic photosynthesis occurred (Figs. 1, 4 and 5, left-hand graphs). In the second phase the photosynthetic rate with sulfide alone becomes increasingly higher than that with sulfide plus DCMU, giving rise to typically Y-shaped time courses in the graphs (Figs. 1 to 4, right). The time at which this change occurred corresponds to 4−5 h after the first sulfide addition. Such responses would be expected if there was a depletion of sulfide under those conditions where photoincorporation was strictly sulfide dependent (with DCMU present) and at the same time the depletion of sulfide without DCMU present would end the direct inhibition of oxygenic photosynthesis, therefore increasing the photoincorporation rates of the treatment without DCMU. In these cases, however, sulfide did not become

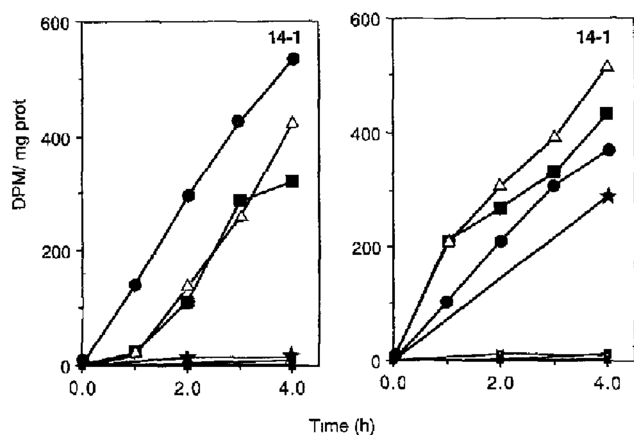


**Fig. 1.** Time course of [$^{14}$C]-$HCO_3^-$ photoincorporation under different conditions for *Oscillatoria amphigranulata* clone 14-1 at 3.8 klx and 40° C. *Left:* Unadapted cells. Sulfide-free control (●); 0.6 mM sulfide initially added (△); 0.6 mM sulfide and 7 μM DCMU initially added (■); 0.6 mM sulfide, 7 mM DCMU and 50 mg $l^{-1}$ CAM initially added (*); other controls at base line: darkened control, darkened plus 0.6 mM sulfide added, 0.6 mM sulfide plus 7 μM DCMU darkened and 7 μM DCMU alone in the light. *Right:* Cells adapted to sulfide by exposure (0.6 mM initially) during 3 h in the light, sparged with $N_2/CO_2$ for 5 min and immediately tested. Additions, legends and controls as in left graph. Sulfide was readded in both sets whenever concentrations dropped below 0.3 mM so as to raise concentrations back to 0.6 mM

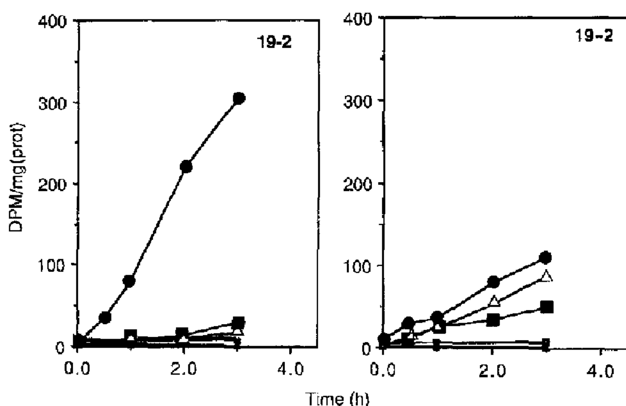


**Fig. 2.** Time course of [$^{14}$C]-$HCO_3^-$ photoincorporation under different conditions for *Oscillatoria amphigranulata* clone 19-2 at 3.8 klx and 40° C. Treatments, additions, legends and controls as in Fig. 1

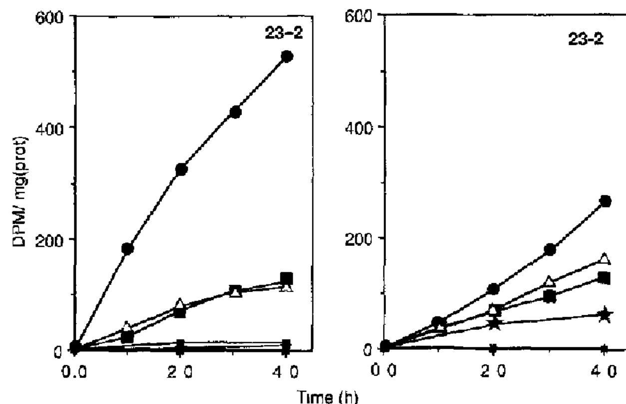


**Fig. 3.** Time course of [$^{14}$C]-$HCO_3^-$ photoincorporation under different conditions for *Oscillatoria amphigranulata* clone 23-2 at 3.8 klx and 40° C. Treatments, additions, legends and controls as in Fig. 1

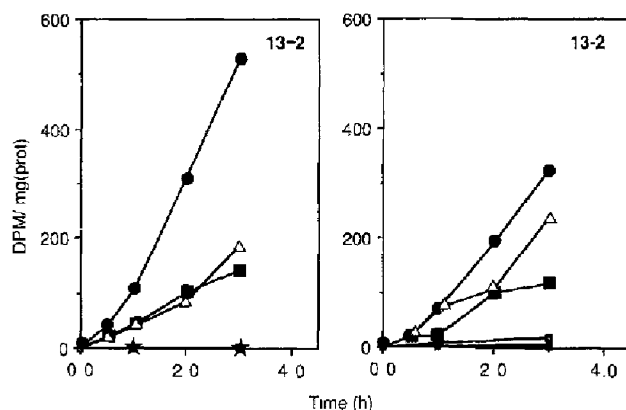


**Fig. 4.** Time course of [$^{14}$C]-$HCO_3^-$ photoincorporation under different conditions for *Oscillatoria amphigranulata* clone 13-2 at 3.8 klx and 40° C. Treatments, additions, legends and controls as in Fig. 1

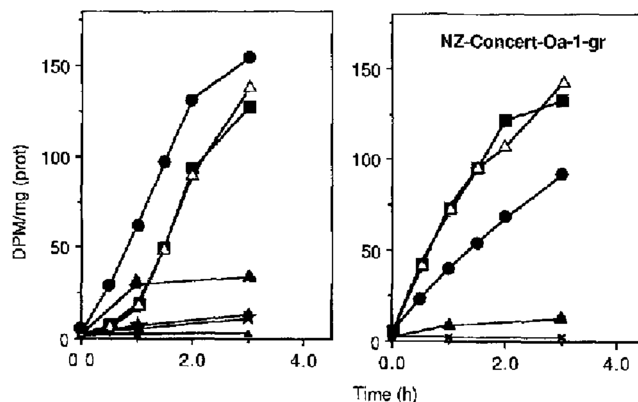


**Fig. 5.** Time course of [$^{14}$C]-$HCO_3^-$ photoincorporation under different conditions for *Oscillatoria amphigranulata* clone NZ-Concert-Oa-1-gr at 3.8 klx and 40° C. Treatments, additions, legends and controls as in Fig. 1

unavailable during experimentation, since vials were replenished with sulfide periodically. Therefore, it is probable that a physiological relief of the initial sulfide inhibition of oxygenic photosynthesis was taking place in the incubations lacking DCMU.

Exposure to sulfide had a long-lasting effect that persisted even after sulfide was removed from the medium. This effect is obvious when comparing the photosynthetic rates of cells never exposed to sulfide to the decreased rates of photoincorporation when sulfide had been removed after exposure. Three hours of exposure to 0.6 mM sulfide (initial concentration), reduced the carbon fixing capacities by 68% in the most sensitive strain and by 30% in the least sensitive (Table 2). Sensitivity judged in this way seems to have no correlation with the levels of sulfide in the original source springs.

However, strong correlation ($R = 0.99$, $n = 7$) was found between the relative capacities for sulfide-dependent photoincorporation and the levels of sulfide of the

**Table 2.** Compared parameters of sulfide tolerance and anoxygenic photosynthesis

| Strain | Rate after exposure[a] (%) | Rate with $H_2S$ and DCMU[b] (%) | Adaptation period (h) | PSII Recovery |
|---|---|---|---|---|
| NZ-Concert-Oa-1-gr | 46 | 81 | 1–2 | – |
| 14-1 | 30 | 74 | 1–2 | – |
| 11-3 | 47 | 42 | n.a. | + |
| 23-2 | 50 | 24 | <0.5 | + |
| 13-2 | 38 | 24 | <0.5 | + |
| 21-3.1 | 51 | 20 | n.a. | + |
| 19-2 | 68 | 27 | 2–3 | + |

[a] % decrease of uptake rate per unit total protein after 3 h exposure to 0.6 mM sulfide and its removal
[b] Rate of uptake in the presence of 0.6 mM sulfide and 7 μM DCMU relative to the uptake rate in the absence of sulfide (non-poisoned)
n.a.: not available

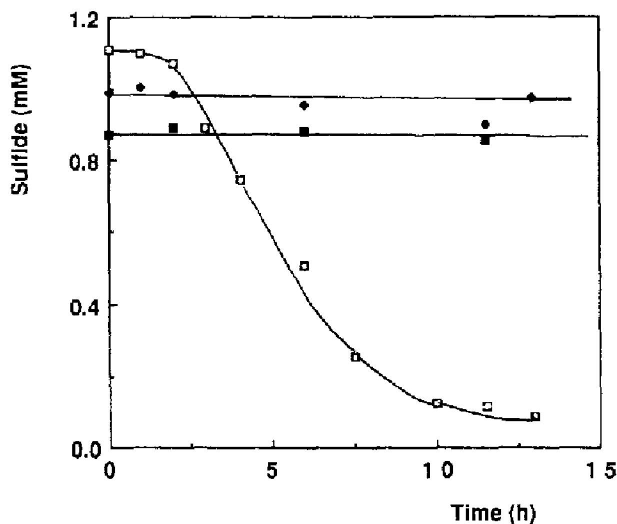


**Fig. 6.** Time course of DCMU-insensitive, light-driven oxidation of sulfide in a batch culture of *Oscillatoria amphigranulata* clone 14-1 at 3.8 klx and 40° C. DCMU (7 μM) and sulfide added to all at time 0. Exposed to light (□); kept in the dark (■); chloramphenicol 50 mg $l^{-1}$ added at time 0 (◆)

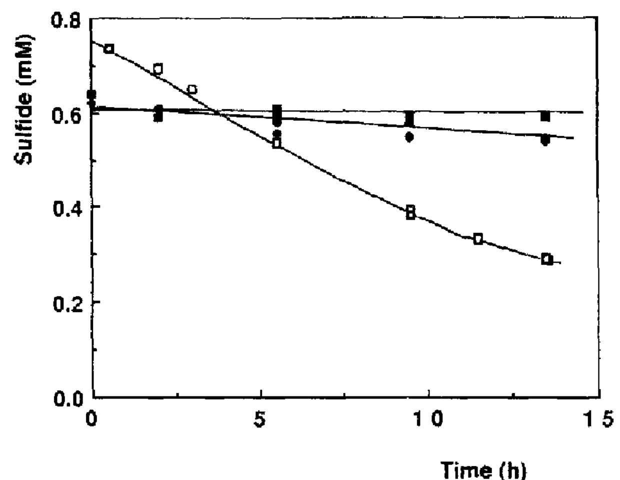


**Fig. 7.** Time course of DCMU-insensitive, light-driven oxidation of sulfide in a batch culture of *Oscillatoria amphigranulata* clone 13-2 at 3.8 klx and 40° C. DCMU (7 μM) and sulfide added to all at time 0. Exposed to light (□); kept in the dark (■); chloramphenicol 50 mg $l^{-1}$ added at time 0 (◆)

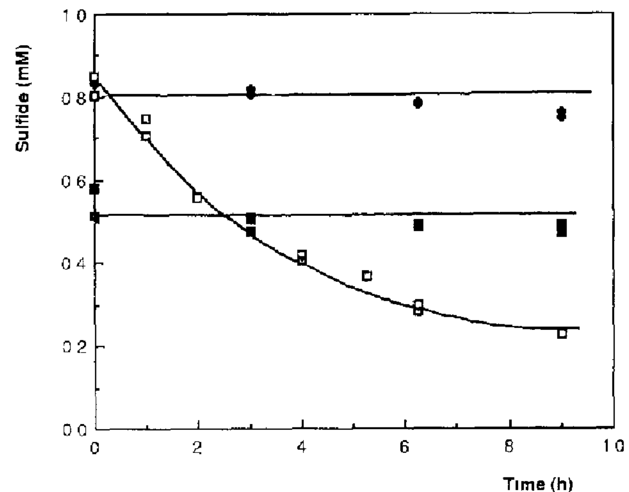


**Fig. 8.** Time course of DCMU-insensitive, light-driven oxidation of sulfide in a batch culture of *Oscillatoria amphigranulata* clone 23-2 at 3.8 klx and 40° C. DCMU (7 μM) and sulfide added to all at time 0. Exposed to light (□); kept in the dark (■); chloramphenicol 50 mg $l^{-1}$ added at time 0 (◆)

source springs. Values for relative anoxygenic photosynthetic rates ranging from 82 to 15% were calculated by dividing the rates of incorporation in the presence of sulfide and DCMU by the respective rate of incorporation with no additions before adaptation (Table 2). This correlation is depicted in Fig. 9.

In $O_2$-free batch cultures in which sulfide and DCMU were added the light-dependent disappearance of sulfide from the medium was measurable and the formation of a fine suspension of elemental sulfur was concomitant with sulfide disappearance in all strains. No thiosulfate beyond trace quantities was detected in the cultures even after sulfide was undetectable (data not shown).

The kinetics of sulfide depletion in the presence of DCMU was closely followed in three strains and the time courses are shown in Figs. 6–8. No sulfide depletion occurred in parallel incubations in the dark. Additional incubations with chloramphenicol added showed, again, no decrease of sulfide. No sulfide depletion occurred in parallel incubations in the dark. The adaptation period prior to sulfide utilization is clearly seen for strain 14-1 (Fig. 6), but again cryptic or not present for strains 13-2 and 23-2 (Figs. 7, 8).

Curve progress data pairs were used to fit an inverse Michaelis-Menten saturation kinetics model through regression analysis of a linearized form of its integrated equation (see Materials and methods). The values found for the affinity constant ($K_m$) are 0.93 mM for strain 13-2, 1.21 mM for strain 23-2 and 1.15 mM for strain 14-1. The values found for the maximum rate of consumption ($V_{max}$) are 2.83, 2.36 and 5.12 μmol mg(prot)$^{-1}$ · $h^{-1}$, respectively. Statistical parameters were: R = 0.90, n = 8 (13-2); R = 0.91, n = 10 (23-2); R = 0.88, n = 7 (14-1).

## Discussion

The results obtained in this survey show that all strains tested, even those isolated from low sulfide springs, are able to perform anoxygenic photosynthesis using sulfide as an electron donor in the fashion that has been described earlier for another isolate of *Oscillatoria amphigranulata* (NZ-Concert-Oa-1, Castenholz and Utkilen 1984a). With the exception of the differences in adaptation periods, all new strains have shown similar physiological traits to those previously reported, although they also showed important quantitative differences in their relative capacity for anoxygenic photosynthesis: 14-1 and NZ-Concert-Oa-1-gr may be regarded as relatively capable strains, whereas 19-2, 13-2, 23-2 and 23-1.1 showed lower relative anoxygenic photosynthetic rates at the same sulfide concentration and 11-3 could be classified as intermediate. As in most cyanobacteria that are capable of facultative anoxygenic photosynthesis (Cohen et al. 1975; Castenholz and Utkilen 1984a; Garlick et al. 1977), this photometabolism was probably restricted to the photo-oxidation of sulfide to sulfur; thiosulfate was not produced and could not be used as electron donor, at least for sulfide adapted cells. Thiosulfate is the oxidized end product of sulfide photooxidation in a strain of *Microcoleus chthonoplastes* (de Wit and van Gemerden 1987). The possibility of sulfur oxidation to sulfate was not tested in our strains; this step is unknown in other facultatively anoxygenic cyanobacteria.

Some differences among strains were found, especially the lag in the time required for physiological responses to occur. The isolates of *O. amphigranulata* (Castenholz and Utkilen 1984a and this work) together with several *Microcoleus chthonoplastes* isolates from around the world (Cohen et al. 1986; de Wit and van Gemerden 1987) constitute a group of cyanobacteria that share the ability to simultaneously perform anoxygenic and oxygenic photosynthesis under relatively high sulfide concentrations. This behaviour also occurs in natural populations of *O. amphigranulata* (Castenholz 1976) and *M. chthonoplastes* (Jørgensen et al. 1986; Javor and Castenholz 1984).

Adaptations to sulfide and anoxygenic photosynthesis have been shown to require protein synthesis as judged by the inhibitory effect of chloramphenicol on the development of the response to sulfide in *O. amphigranulata* (Castenholz and Utkilen 1984a). Adaptation periods of about 2 h for anoxygenic photosynthesis have also been found for *Oscillatoria limnetica* (Oren and Padan 1978) and *Microcoleus chthonoplastes* (3 h) (de Wit and van Gemerden 1987). In *O. limnetica* the potent reducing agent sodium dithionite has been shown to relieve adaptation time for anoxygenic photometabolism (Belkin and Padan 1983), but this seems not to be the case in *O. amphigranulata* (Castenholz and Utkilen 1984a). In the course of the present experiments, the inhibitory effects of chloramphenicol were clear, although in some cases (Table 2) the initial adaptation period was not present or else shorter than the time of the initial sampling intervals. The adaptation periods ($< 0.5$ h $-$ 3 h) were neither correlated with the levels of sulfide in the source springs nor to the total photoincorporation rate in the absence of sulfide, and may simply be the result of differences in rates of protein synthesis or in the degree of poisoning of other cellular components by sulfide.

In the new *O. amphigranulata* strains, a relatively long adaptation period (4 – 5 h) in the presence of sulfide was necessary for PII to show signs of recovery from initial inhibition. After this period, photoincorporation in the absence of sulfide became increasingly higher than that in the presence of sulfide plus DCMU (Figs. 1 to 4). In one strain (NZ-Concert-Oa-1-gr, Fig. 5), this was not obvious even after six hours. Castenholz and Utkilen (1984a) showed that this response occurred only under high light (9.6 klx) in NZ-Concert-Oa-1, but was not detected for the 2 – 3 h of incubation at 3 – 4 klx, an intensity equivalent to that used in our study (3.8 klx).

The relative rates of anoxygenic photosynthetic carbon assimilation show striking differences among strains. The highest rates (81%, 74%) are comparable to those found for *M. chthonoplastes*, *O. limnetica* and another hot spring *Oscillatoria* (in the range of 70%) by Cohen et al. (1986) at the same sulfide concentration. A study of the effects of sulfide concentration on the relative rates of photoincorporation was not made, but all strains showed decreased responses at sulfide concentrations above 2 mM (data not shown). Rather, all strains were submitted to a common concentration of 0.6 mM sulfide. This concentration was not necessarily that at which rates of anoxygenic photosynthesis were maximal for each strain but, nevertheless, offers a reference point at which differences in performance can be compared. From our data it can be seen that strains isolated from low sulfide springs (lower than a constant 0.6 mM) do show a relatively lower rate than those isolated from high sulfide springs.

For the strains tested (two from low sulfide springs and one from high sulfide springs), the analysis of the kinetic characteristics of sulfide utilization revealed the low affinity for sulfide of the system regardless of the strain origin. Affinity constants ($K_m$) at about 1 mM were estimated for the three strains. It can be calculated that such a system theoretically would be operating at nearly maximum velocity at concentrations of sulfide that are poisonous to the cell metabolism (see Fig. 10). Very similar values for $K_m$ of photooxidation of sulfide to thiosulfate were estimated in a strain of *M. chthonoplastes* (0.97 mM) in pure culture (de Wit and van Gemerden 1987), even though its growth on sulfide was halted at concentrations of 1 mM or more (de Wit et al. 1988). In our case, estimated values for $V_{max}$ show significant differences, with a "rate" for the "high sulfide" strain almost double than that of the "low sulfide" strains. $V_{max}$ estimated for *M. chthonoplastes* (de Wit and van Gemerden 1987) was about 2.6 µmol mg(prot)$^{-1}$ h$^{-1}$, which is comparable to our low sulfide isolate results. However, since the *M. chthonoplastes* value was obtained under low-light limitation, it could be expected to increase with light saturating conditions.

The correlation between the anoxygenic photosynthetic rates of sulfide-adapted cells and the sulfide

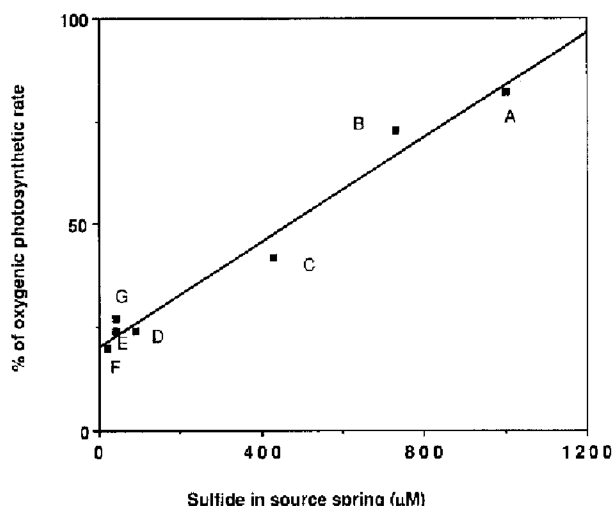


**Fig. 9.** Relation between the rates of anoxygenic photosynthesis at 0.6 mM sulfide in sulfide-adapted cells expressed as percentage of oxygenic photosynthetic rate (never exposed to sulfide) and the sulfide concentration in the respective source spring water at the edge of the *Oscillatoria amphigranulata* mat at the time of collection of each strain. *A:* NZ-Concert-Oa-1-gr, *B:* 14-1, *C:* 11-3, *D:* 23-2, *E:* 13-2, *F:* 21-3.1, *G:* 19-2

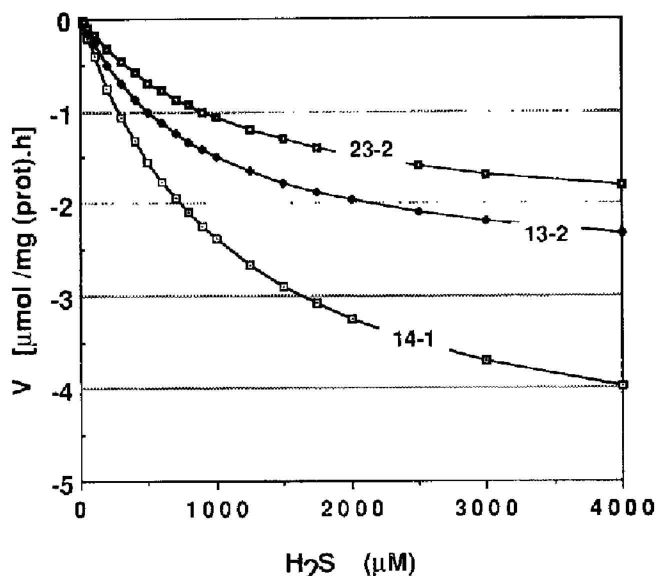


**Fig. 10.** Theoretical Michaelis-Menten saturation kinetics calculated for three strains. *Y-axis:* Velocity of sulfide change in the medium. *X-axis:* concentration of substrate (sulfide). Note that in all cases $V_{max}$ is attained at concentrations over 2 mM sulfide

content in the spring waters of origin (Fig. 9) clearly speaks for a finely-tuned adaptation in terms of this capacity in natural populations. Sulfide levels in the natural habitats of *O. amphigranulata* are substantially different for the distinct populations, ranging from under 0.1 mM up to about 2.2 mM (Castenholz 1976; this work). At the alkaline pH levels of these springs the most membrane permeable form of the sulfide equilibrium in water ($H_2S$) is restricted, making the probable toxicity of sulfide less extreme (see Howsley and Pearson 1979). The geothermal (primary) origin of sulfide in the spring waters results in fairly constant levels, although actual concentrations in well-developed microbial mats may be influenced by sulfide biogenesis and consumption, which results in steep vertical gradients within the mats. Physically separated populations of this cyanobacterium, thriving in different hot springs have been exposed to constant but different concentrations of sulfide for prolonged periods. These particular conditions create an environment suited for a finely tuned adaptation. The sulfide concentration in the bathing water should be of paramount importance in the processes of colonization or re-colonization, since it is at this time when variations in sulfide concentrations due to biogenesis and consumption do not exist and the colonizing organisms are exposed directly to the physico-chemical conditions of spring water. The differential capacities for anoxygenic photosynthesis were preserved during nearly two years of aerobic laboratory culture (17 years for NZ-Concert-Oa-1-gr), and in some cases have persisted through repeated cloning. Only on one occasion was a variant isolated by recloning that had lost its abilities to adapt to sulfide (Castenholz and Utkilen 1984b). This testifies to a high degree of conservation of the sulfide-tolerance characteristics acquired in nature.

Conversely, in 5 strains of *M. chthonoplastes* from widely different geographic locations, "virtually identical" responses were found in the relative rates of radioactive carbon photoincorporation in the presence of sulfide and DCMU (Cohen et al. 1986). During the crucial times of colonization in marine sediments, little or no sulfide may be present. It is also known that the solely biogenic origin of sulfide in the typical, well-developed coastal mats inhabited by *Microcoleus* does not give a continuous exposure to narrow concentration range, because diel transitions occur in the mat surface between oxygen super-saturation and anoxic, sulfide rich conditions (Jørgensen et al. 1986). In *Microcoleus*, therefore, the homogeneity of the responses may simply reflect the similarity of certain aspects of the habitats in different geographical locations, such as sulfide concentration fluctuations.

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