

## Phototropic sulfur and sulfate-reducing bacteria in the chemocline of meromictic Lake Cadagno, Switzerland

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### ABSTRACT

Lake Cadagno, a crenogenic meromictic lake located in the catchment area of a dolomite vein rich in gypsum in the Piora Valley in the southern Alps of Switzerland, is characterized by a compact chemocline with high concentrations of sulfate, steep gradients of oxygen, sulfide and light and a turbidity maximum that correlates to large numbers of bacteria (up to  $10^7$  cells ml<sup>-1</sup>). The most abundant taxa in the chemocline are large- and small-celled purple sulfur bacteria, which account for up to 35% of all bacteria, and sulfate-reducing bacteria that represent up to 23% of all bacteria. Depending on the season, as much as 45% of all bacteria in the chemocline are associated in aggregates consisting of different populations of small-celled purple sulfur bacteria of the genus *Lamprocystis* (up to 35% of all bacteria) and sulfate-reducing bacteria of the family *Desulfobulbaceae* (up to 12% of all bacteria) that are almost completely represented by bacteria closely related to *Desulfocapsa thiozymogenes*. Their association in aggregates is restricted to small-celled purple sulfur bacteria of the genus *Lamprocystis*, but not obligate since non-associated cells of bacteria related to *D. thiozymogenes* are frequently found, especially under limited light conditions in winter and early summer. Aggregate formation and concomitant growth enhancement of isolates of both partners of this association suggests synergistic interactions that might resemble a sulfide-based source-sink relationship between the sulfate-reducing bacterium that is able to sustain growth by a disproportionation of inorganic sulfur compounds (sulfur, thiosulfate, sulfite), with the purple sulfur bacteria acting as a biotic scavenger. The availability of these isolates opens up the door for future studies considering other facets of potential interactions in aggregates since both types of organisms are metabolically highly versatile and interactions may not be limited to sulfur compounds only.

*Key words:* fluorescent in situ hybridization (FISH), meromixis, molecular ecology, rRNA, stratification

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### 1. INTRODUCTION

Meromictic lakes are characterized by permanent stratification due to incomplete circulation resulting in a portion of water mass that never mixes with the rest of the water body (Wetzel 1983). The water column of meromictic lakes is generally characterized by three distinct layers: the mixolimnion, the upper oxic layer that is usually characterized by complete circulation during the year, the monimolimnion, the anoxic lower layer that is characterized by a stagnant water body, and the chemocline, a compact transition zone between mixo- and monimolimnion usually characterized by steep, but relatively stable physico-chemical gradients (Sorokin 1970; Lindholm 1987; Pedrós-Alió & Guerrero 1993; van Gemerden & Mas 1995; Overmann *et al.* 1996). The physico-chemical conditions in the chemocline support growth of large numbers of microorganisms with different physiological groups substituting each other at small depth intervals along the vertical gradients (Jørgensen *et al.* 1979; Guerrero *et al.* 1985; Overmann *et al.* 1991). The compactness of the chemocline facilitates accurate sampling, a necessary prerequisite for reliable determination of physico-chemical characteristics and microbial community structure (Tonolla *et al.* 2000, 2003; Peduzzi *et al.* 2003); the chemoclines

of permanently stratified lakes are therefore optimal systems for ecological studies of aquatic microorganisms along vertical environmental gradients.

During the last decade, the chemocline of Lake Cadagno, Switzerland has been the object of many ecological studies on microorganisms. Lake Cadagno is a crenogenic meromictic lake located 1923 m above sea level in the Piora valley in the southern Alps of Switzerland (46°33' N, 8°43' E) with a surface area of  $26 \times 10^5$  m<sup>2</sup> and a maximum depth of 21 m. Permanent stratification is stabilized by density differences between the mixolimnion characterized by electrolyte-poor surface water, and the monimolimnion containing water of high ionic strength (9-10 mM) constantly supplied by subaquatic springs (Lehmann *et al.* 1998; Tonolla *et al.* 1998; Del Don *et al.* 2001). Lake Cadagno's mixolimnion extends to a depth of 10-12 m, and is characterized by low nutrient concentrations with phosphate close to the detection limit ( $<1 \mu\text{g l}^{-1}$ ), and nitrate concentrations below  $50 \mu\text{g l}^{-1}$  (Tonolla *et al.* 1998, 1999). The monimolimnion contains higher nutrient concentrations with phosphate concentrations up to  $400 \mu\text{g l}^{-1}$  and ammonia concentrations up to  $5 \text{ mg l}^{-1}$ . The chemocline transition between the oxic mixolimnion and anoxic monimolimnion is characterized by vertical gradients of these nutrients, but also of different organic and

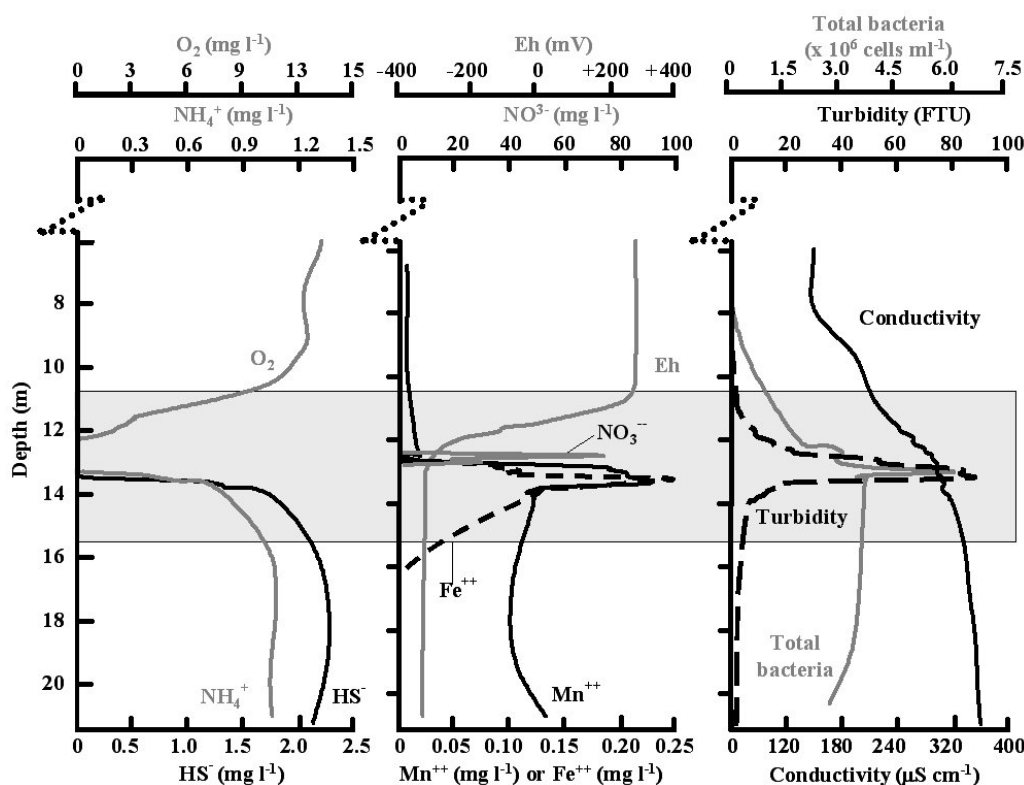


Fig. 1. Characteristic physico-chemical conditions in Lake Cadagno, Switzerland (Tonolla *et al.* 1998). The chemocline is marked by the darkened area.

inorganic electron donors and acceptors as well as light intensity (Fig. 1) (Tonolla *et al.* 1988, 1998; Del Don *et al.* 2001).

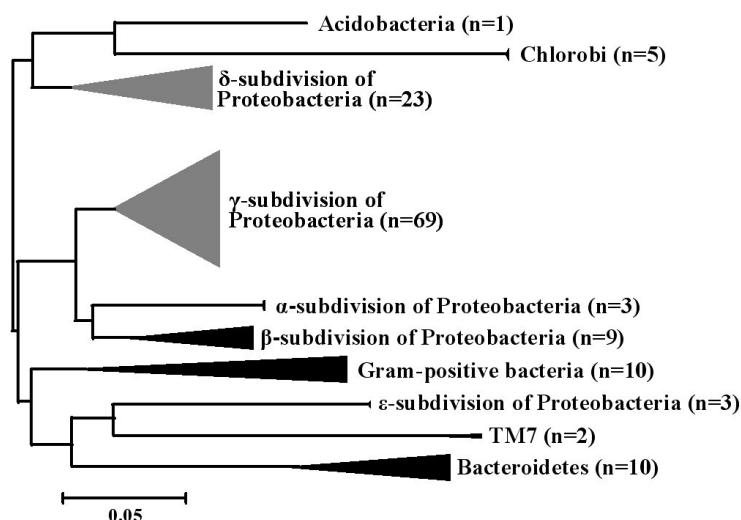
Due to the infiltration of water through a dolomite vein rich in gypsum, Lake Cadagno contains approximately 10 to 20 times more sulfate than most freshwater lakes with concentrations in the chemocline and monimolimnion ranging from 120 to 160 mg l<sup>-1</sup> (Hanselmann & Hutter 1998; Lehmann *et al.* 1998). The basic physico-chemical conditions in the chemocline of Lake Cadagno are therefore dominated by compounds of the sulfur cycle such as sulfate and sulfide (Tonolla *et al.* 1998; Wagener *et al.* 1990) with gradients relatively stable during the year (Tonolla *et al.* 1998, 2003). Major changes in physico-chemical conditions are only displayed for light intensity that is more than 1 order of magnitude higher during summer (up to 5.8 μE m<sup>-2</sup> s<sup>-1</sup>) than during winter (0.3 μE m<sup>-2</sup> s<sup>-1</sup>). Within the chemocline, light intensities usually decrease with depth by up to 2 orders of magnitude (Tonolla *et al.* 2003).

## 2. MICROBIAL COMMUNITY STRUCTURE

Annual shifts in light intensity correlate with changes in turbidity that is highest in summer (up to 57 Formazine Turbidity Units (FTU)), lowest during winter (<8 FTU) and increasing after melting of the snow and ice cover (16 FTU) (Tonolla *et al.* 2003). The turbidity values in the chemocline correlate with cell densities of bacteria, with maximum numbers of up to 10<sup>7</sup> cells ml<sup>-1</sup>

(Fig. 1) (Tonolla *et al.* 1999; Lüthy *et al.* 2000; Camacho *et al.* 2001). Highest bacterial numbers are generally found at depths where sulfide becomes detectable and concentrations increase with depth to up to 3–8 mg l<sup>-1</sup> (Fig. 1) suggesting the presence of both sulfide-producing as well as sulfide-consuming populations in the bacterial plume. This is supported by *in situ* analyses of sulfide turnover rates showing net sulfide consumption during the day and net production during the night during maximal seasonal turbidity (Lehmann *et al.* 1998; Lüthy *et al.* 2000). Net day-time sulfide consumption is attributed to the large activity of phototrophic sulfur bacteria, while nocturnal net sulfide production may be due to increased activity of sulfate-reducing bacteria and anaerobic respiration of internal storage compounds of phototrophic bacteria (Del Don *et al.* 1994; Mas & van Gemerden 1995).

Based on their distinct morphologies and their intensive autofluorescence, populations of phototrophic sulfur bacteria were generally characterized as purple sulfur bacteria, more specifically as large-celled *Chromatium okenii* with an average cell size of 4.5–6 × 8–15 μm and as small-celled *Amoebobacter purpureus* (recently reclassified as *Lamprocystis purpurea*; Imhoff 2001) with an average cell size of 3.3–3.8 × 3.5–4.5 μm (Peduzzi *et al.* 1993; Fischer *et al.* 1996). Together these resemble nearly 35% of all bacteria in the chemocline of Lake Cadagno. Sulfate-reducing bacteria account for up to 23% of the total bacterial community (Peduzzi *et al.*



**Fig. 2.** Neighbor Joining Tree showing the assignment of clones from the 16S rRNA gene clone library of the chemocline of Lake Cadagno to higher phylogenetic groups of Bacteria. The purple sulfur and sulfate-reducing bacteria can be found within the  $\gamma$ - and  $\delta$ -subdivision of Proteobacteria, respectively.

2003), and are represented by the families Syntrophaceae and Desulfobulbaceae (according to the new taxonomic outlines, Garrity *et al.* 2003), 9 and 14% of the total bacterial community, respectively, depending on the season and averaged over the whole chemocline (Tonolla *et al.* 2000; Peduzzi *et al.* 2003).

The importance of purple sulfur and sulfate-reducing bacteria in the chemocline of Lake Cadagno is reflected in a gene clone library that harbored PCR-amplified, almost complete 16S rRNA genes of uncultured bacteria of the chemocline (Demarta *et al.* 1998). More than 50% of the 129 clones analyzed were assigned to the  $\gamma$ -subdivision of Proteobacteria that include the purple sulfur bacteria, and 13% to the  $\delta$ -subdivision of Proteobacteria that contain many sulfate-reducing bacteria. Additional clones belong to the green sulfur bacteria (4%), the  $\beta$ -subdivision of Proteobacteria (7%), the Firmicutes (8%), the Bacteroidetes (8%), bacteria of the yet uncultured phylum TM7 (2%), and the Acidobacteria (1%) (Fig. 2). Except for the  $\alpha$ -subdivision of Proteobacteria, these data correlate well with cell numbers obtained by *in situ* hybridization that reveal that the bacterial community in the chemocline of Lake Cadagno mainly consists of Proteobacteria with numbers for the  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -subdivision of Proteobacteria accounting for 23, 17, 45 and 15% of the total number of bacteria, respectively (Tonolla *et al.* 1998, 1999).

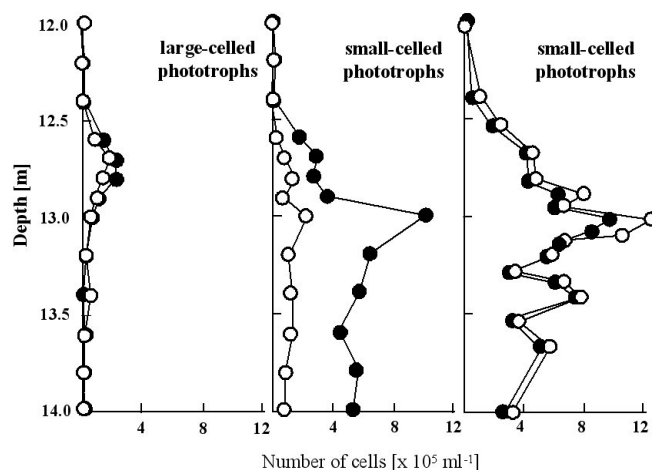
The large number of sequences in the 16S rRNA gene clone library with 67% of all clones potentially representing phototrophic (i.e. purple and green) sulfur and sulfate-reducing bacteria, and the prominent numerical abundance of cells identified as purple sulfur or sulfate-reducing bacteria (50% of all cells) prompted more detailed analyses of these bacterial groups in the chemocline of Lake Cadagno. These analyses revealed much about the genetic diversity and distribution of these bacteria in the chemocline, and provided informa-

tion on potential interactions between populations under different environmental conditions (Tonolla *et al.* 1999, 2000, 2003; Peduzzi *et al.* 2003a; b).

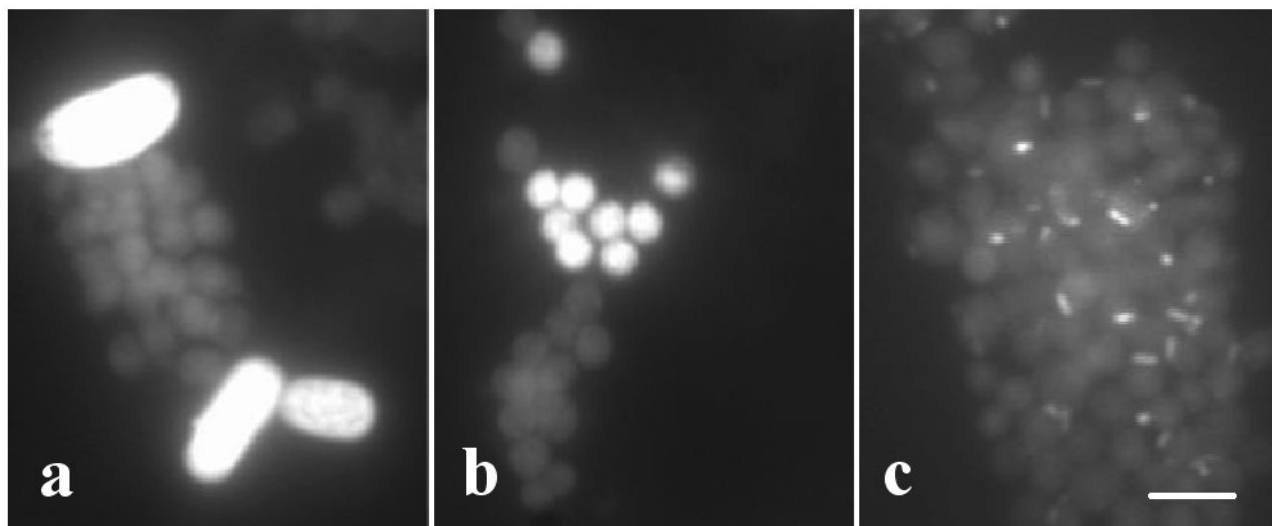
### 3. PHOTOTROPIC SULFUR BACTERIA IN THE CHEMOCLINE

Comparative sequence analysis of the 16S rRNA gene clone library suggests a relatively limited diversity of phototrophic sulfur bacteria in the chemocline of Lake Cadagno. Two clones contained 16S rRNA genes resembling those of *C. okenii* and *L. purpurea*, respectively, thus confirming the assumption on their presence based on morphological criteria (Tonolla *et al.* 1999). In addition, however, sequences of three groups of purple sulfur bacteria related to *L. purpurea* and *L. roseopersicina* (Tonolla *et al.* 1999), and two groups clustering with *Thiocystis minor* and *T. gelatinosa*, respectively (Tonolla, unpublished data) were retrieved. Sequences representing green sulfur bacteria that were found in much smaller numbers in the library (4% for Chlorobi) were identified as those of *Chlorobium phaeobacteroides* (Tonolla *et al.* 2003) and *Chlorobium clathratiforme* (Tonolla, unpublished data), respectively.

All large-celled purple sulfur bacteria in the chemocline of Lake Cadagno are identified as *C. okenii* by *in situ* hybridization with specific probes (Fig. 3, 4) (Tonolla *et al.* 1999). *C. okenii* is usually detected at the upper border of the chemocline, where anoxic conditions and high sulfate and low sulfide concentrations are prevalent, with maximum numbers (approx.  $50 \times 10^3$  cells  $\text{ml}^{-1}$ ) comparable to levels in other lakes such as lake Belovod (Sorokin 1970). Although cells of *C. okenii* represent only approximately 0.1-0.2% total bacterial number (Tonolla *et al.* 1999), they account for up to 40% of the total biomass due to their large biovolume ( $55.8 \pm 3.6 \mu\text{m}^3 \text{ cell}^{-1}$ ) (Tonolla *et al.* 1998).



**Fig. 3.** Distribution profiles of large- and small-celled purple sulfur bacteria in the chemocline of Lake Cadagno. Cell numbers were determined according to morphotypes (closed circles) and after *in situ* hybridization with specific probes (open circles) (probe Cmok453 targeting large-celled *C. okenii* [left panel]; Apur453 targeting small-celled *A. purpureus* [middle panel]; and a combination of Apur453, Laro453, S453D and S453F targeting all clones resembling small-celled purple sulfur bacteria retrieved from Lake Cadagno [right panel]). Circles represent means of 40 counts. Standard errors have been omitted from the presentation for clearer resolution.



**Fig. 4.** *In situ* detection of large-celled purple sulfur bacterium *C. okenii* after hybridization with probe Cmok453 (a), small-celled purple sulfur bacterium *A. purpureus* after hybridization with probe Apur453 (b), and sulfate-reducing bacterium *D. thiozymogenes* after hybridization with probes DSC213 and DSC441 (c) in the chemocline of Lake Cadagno. Bar represents 10  $\mu\text{m}$ .

These high values are only encountered in October at the end of the year (Tonolla *et al.* 2003), suggesting *C. okenii* might be more competitive at shorter day lengths. Indeed, long dark periods have been reported to favor growth of large-celled purple sulfur bacteria in competition with small-celled purple sulfur bacteria (van Gemerden 1974).

Small-celled purple sulfur bacteria in the chemocline of Lake Cadagno are largely represented by four major populations belonging to the genus *Lamprocystis*. The most abundant are populations D and F (40–80% depending on the season) that are not represented by a cultured relative (Tonolla *et al.* 1999), while *L. pur-*

*purea* and *L. roseopersicina* are found in numbers about one order of magnitude lower (Tonolla *et al.* 2003). Populations D and F are slightly smaller in size with biovolumes of  $7.82 \pm 0.37$  and  $6.77 \pm 0.35 \mu\text{m}^3 \text{ cell}^{-1}$ , respectively, than *L. purpurea* and *L. roseopersicina* which have biovolumes of about  $8.45 \pm 0.65 \mu\text{m}^3 \text{ cell}^{-1}$  (Tonolla *et al.* 1999). All populations are relatively evenly distributed throughout the entire chemocline during winter and spring, while a clear microstratification is exhibited during summer, suggesting specific eco-physiological adaptations of these populations to the steep physico-chemical gradients in the chemocline of Lake Cadagno (Tonolla *et al.* 2003).

Green sulfur bacteria in the chemocline are entirely represented by *Ch. phaeobacteroides* between years 1994 and 2000, with numbers generally comparable to those of *C. okenii*, *L. purpurea* or *L. roseopersicina*, but about one order of magnitude lower than the dominant populations D and F (Tonolla *et al.* 2003). Though generally detectable throughout the season and at all depths, due to the small numbers and sizes (average biovolume of  $0.8 \pm 0.1 \mu\text{m}^3 \text{ cell}^{-1}$ ), cells of *Ch. phaeobacteroides* never constitute an important part of the bacterial biomass, accounting for less than 1% of total biomass averaged over the whole chemocline (Tonolla *et al.* 2003). Recently, *Ch. phaeobacteroides* has been displaced by a rapidly increasing population of *Ch. clathratiforme* that also reduced numbers of purple sulfur bacteria, presumably as a consequence of an unusually deep mixing event during the fall of 1999 that disrupted the chemocline and likely disturbed the nutrient balance in the chemocline (Tonolla, unpublished data). In an undisturbed chemocline, however, purple sulfur bacteria are the dominant group of phototrophic sulfur bacteria.

The dominance of purple sulfur bacteria over green sulfur bacteria in the chemocline might be due to better adaptations to the physico-chemical conditions, but also to their metabolic versatility. Green sulfur bacteria are strictly anaerobic, obligate phototrophs, while some purple sulfur bacteria can tolerate higher redox potentials, oxygen and sulfide concentrations compared to green sulfur bacteria (Guerrero *et al.* 1987; Vila *et al.* 1998). In addition, purple sulfur bacteria are more versatile with respect to carbon resources (Trüper 1981; van Gernerden & Mas 1995), may be able to simultaneously oxidize sulfide and polysulfide (van Gernerden 1987) and have the capacity to grow chemolithotrophically (Eichler & Pfennig 1988; Schaub & van Gernerden 1994). In addition, purple sulfur bacteria such as gas-vacuolated *L. purpurea*, *L. roseopersicina* or flagellated *C. okenii* are motile, and have the potential to reposition under changing environmental conditions unlike non-motile green sulfur bacteria such as *Ch. phaeobacteroides*.

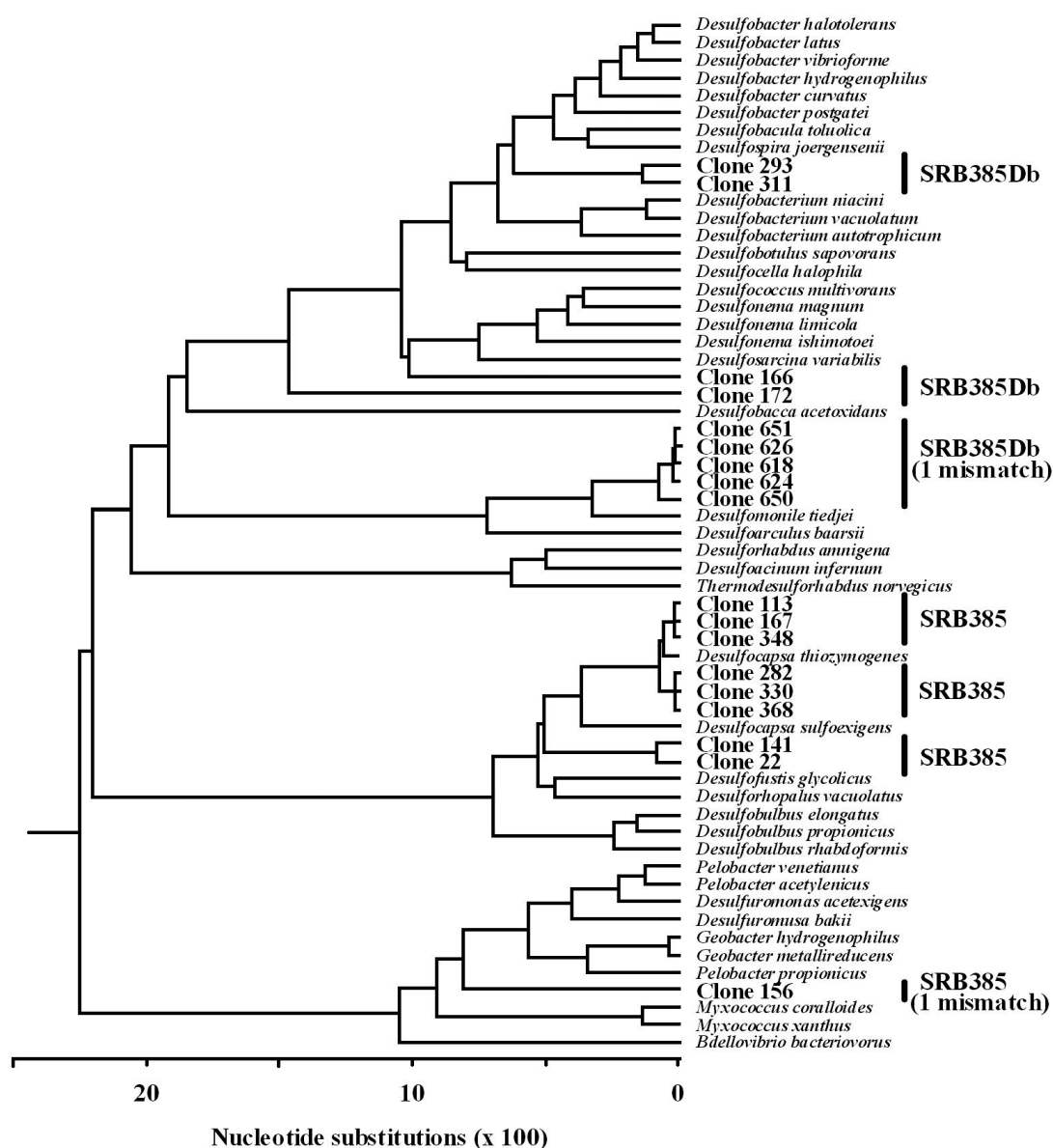
Another advantage purple sulfur bacteria may have over green sulfur bacteria is the ability of all small-celled purple sulfur bacteria to form aggregates consisting of 200 to 900 cells (Tonolla *et al.* 1999, 2003). Bacterial aggregates might provide micro-environmental conditions with more favorable basic growth conditions such as nutrient- or electron acceptor-availability for individual cells inside the aggregate compared to external conditions, or those experienced by a free-living organism (Schramm *et al.* 1999). Aggregation is also considered a defense against adverse environmental conditions such as oxygen exposure of anaerobic bacteria (Cypionka 2000). Aggregate formation among sulfate-reducing bacteria has been recently reported for various sulfidogenic, oxygen-exposed environments (Fukui & Takii 1990; Krekeler *et al.* 1997; Manz *et al.* 1998; Minz *et al.* 1999; Teske *et al.* 1998;

Wieringa *et al.* 2000), and has been related to increased tolerance to oxygen (Brune *et al.* 2000; Cypionka 2000) and more positive redox potentials (Manz *et al.* 1998). Also, associations create relatively stable micro-environmental conditions in habitats where bioturbation phenomena or continuous movement of the plume due to chemo- or phototaxis result in rapid changes of environmental conditions (e.g. intensity of light, sulfide concentrations) (Egli *et al.* 1998; Hanselmann & Hutter 1998; Lüthy *et al.* 2000). Thus, aggregate formation may result in enhanced environmental resiliency compared to the single-cell status.

#### 4. SULFATE-REDUCING BACTERIA IN THE CHEMOCLINE

Comparative sequence analysis of clones positive to probes SRB385 and SRB385Db in the 16S rRNA gene clone library reveals the presence of three groups, with most clones representing organisms closely related to *Desulfomonile tiedjei* (Syntrophaceae) (Fig. 5). Clones representing members of the Desulfobulbaceae form two distinct clusters (Fig. 5), with the most prominent cluster being closely related to *Desulfocapsa thiozymogenes* (Tonolla *et al.* 2000). The second cluster comprises bacteria only distantly related to any cultured bacteria such as *Desulfofustis glycolicus*, *D. thiozymogenes*, *D. sulfoexigens*, and *Desulforhopalus vacuolatus* (Tonolla *et al.* 2000). Profiles of cell numbers of the Syntrophaceae and Desulfobulbaceae in the chemocline correspond roughly to each other and to the turbidity profiles over an annual cycle, however, Desulfobulbaceae are most prominent at the end of the summer ( $13 \pm 2\%$  of all bacteria compared to Syntrophaceae with  $5 \pm 1\%$ ) while numbers of the Syntrophaceae are much higher than those of the Desulfobulbaceae at the beginning of the summer ( $14 \pm 2\%$  and  $3 \pm 1\%$ , respectively; Tab. 1) (Peduzzi *et al.* 2003).

Cells populations of the Syntrophaceae and Desulfobulbaceae are essentially represented by *D. tiedjei* and *D. thiozymogenes*, respectively. Cells related to *D. tiedjei* display a distinctive morphology, and resemble bacteria previously described as "morphotype R" (Bensadoun *et al.* 1998). "Morphotype R" was proposed to be related to "morphotype T5" (Caldwell & Tiedje 1975; Bensadoun *et al.* 1998) and its cultured representatives *D. tiedjei* and *D. liminaris* (DeWeerd *et al.* 1990; Sun *et al.* 2001). However, these cells are more important in the monimolimnion, where they represent up to 44% of all bacteria, than in the chemocline (Tonolla, unpublished data). The most numerous sulfate-reducing bacteria in the chemocline are related to *D. thiozymogenes*, and account for up to 99% of the Desulfobulbaceae. These results suggest different eco-physiological adaptations to environmental characteristics of the bacteria related to *D. tiedjei* and to *D. thiozymogenes*, respectively.



**Fig. 5.** Neighbor-joining tree depicting the phylogenetic position of clones from the 16S rRNA gene clone library from the chemocline of Lake Cadagno that have been detected after *in situ* hybridization with probes SRB385 and SRB385Db.

**Tab. 1.** Seasonal shift in abundance of sulfate-reducing bacteria (% of DAPI-stained cells, SE in brackets) in the chemocline of Lake Cadagno.

|                                                                 | October '98 | March '99  | June '99   | August '99 |
|-----------------------------------------------------------------|-------------|------------|------------|------------|
| DSC441 + DSC213 (attached)                                      | 12.3 (1.6)  | 6.6 (0.7)  | 2.6 (0.3)  | 6.0 (0.5)  |
| DSC441 + DSC213 (free)                                          | 0.4 (0.0)   | 1.5 (0.3)  | 1.6 (0.2)  | 0.3 (0.1)  |
| SRB441 (free)                                                   | 1.2 (0.2)   | 0.2 (0.0)  | 0.2 (0.0)  | 0.1 (0.0)  |
| SRB385                                                          | 12.9 (1.7)  | 8.7 (0.9)  | 3.4 (0.3)  | 5.8 (0.5)  |
| SRB385Db                                                        | 4.7 (0.6)   | 3.9 (0.4)  | 14.9 (1.5) | 7.5 (0.6)  |
| Sulfate-reducing bacteria (SRB385+SRB385Db)                     | 17.6 (2.3)  | 12.6 (1.3) | 18.3 (1.0) | 13.3 (1.1) |
| Small-celled purple sulfur bacteria                             | 35.0 (4.5)  | 19.5 (2.2) | 14.5 (1.4) | 27.5 (2.3) |
| Small-celled purple sulfur bacteria + sulfate reducing bacteria | 52.6 (6.8)  | 32.1 (3.5) | 32.8 (3.2) | 40.8 (3.4) |

Since isolates of "morphotype R" are not yet available and phylogenetic relationships do not necessarily reflect physiological relationships (Pace 1999; Achenbach & Coates 2000; Zinder & Salyers 2001), a discussion about potential properties of "morphotype R" and its potential function in Lake Cadagno remains highly speculative. Although "morphotype R" was detected under similar environmental conditions and is morphologically similar and phylogenetically related to *D. tiedjei*, a further characterization and comparison of "morphotype R" with *D. tiedjei* which is a sulfate reducing bacterium that can also grow by reductive dehalogenation of chlorinated organic compounds such as 3-chlorobenzoate (DeWeerd *et al.* 1990; Sun *et al.* 2001) requires pure culture studies.

A pure culture is available from the chemocline of Lake Cadagno that resembles *D. thiozymogenes* although it differs from the type strain (Janssen *et al.* 1996) by its ability to grow on lactate and pyruvate (Peduzzi *et al.* 2003). Like *D. thiozymogenes*, this isolate is able to disproportionate inorganic sulfur compounds (sulfur, thiosulfate, sulfite) and to grow, although growth on sulfur requires a sulfide scavenger such as amorphous ferric hydroxide (FeOOH), generally resulting in the formation of sulfate along with iron sulfides (Thamdrup *et al.* 1993; Lovely & Phillips 1994), and thus removing free sulfide from the culture (Janssen *et al.* 1996). Disproportionation of elemental sulfur to sulfate and sulfide is considered a novel form of anaerobic respiration with environmental relevance (Lovely & Coates 2000). The detection of large numbers of sulfur disproportionating bacteria in marine sediments over a wide range of environmental conditions was used to advocate a prominent role of these organisms in these environments (Canfield *et al.* 1998). Following this line of argumentation, the prominent abundance of bacteria related to *D. thiozymogenes* in the chemocline of Lake Cadagno suggests a certain importance of sulfur disproportionation in the biogeochemical sulfur cycle in Lake Cadagno, similar to marine and freshwater sediments (Jørgensen 1990a, b; Bak & Pfennig 1991; Canfield & Thamdrup 1994; Canfield *et al.* 1998).

##### 5. INTERACTIONS BETWEEN PURPLE SULFUR AND SULFATE-REDUCING BACTERIA IN THE CHEMOCLINE

Small-celled purple sulfur bacteria of the genus *Lamprocystis* and sulfate-reducing bacteria related to *D. thiozymogenes* are generally found in associated aggregates within the chemocline of Lake Cadagno, a finding not yet been reported for purple sulfur and sulfate-reducing bacteria in other natural environments (e.g. Pfennig 1980; Pedrós-Alió & Guerrero 1993; van Gernerden & Mas 1995; Overmann 1997; Overmann & van Gernerden 2000; Overmann & Schubert 2002). This association is not obligate, but promoted by environmental conditions. During summer, associated cells ac-

counted for up to 97% of all cells of bacteria related to *D. thiozymogenes*, a number decreasing to about 62% during winter and spring (Tonolla *et al.* 2000). This decrease is related to a lower abundance of small-celled purple sulfur bacteria that account for only up to 20% of all bacteria and form small aggregates (approx. 5-40 cells) during winter. Much larger numbers representing up to 35% of all bacteria and larger aggregates (approx. 200-900 cells) are encountered during summer (Tab. 1) (Peduzzi *et al.* 2003). *D. thiozymogenes*-related bacteria do not specifically associate with one of the populations of small-celled purple sulfur bacteria although these display different distribution profiles in the chemocline suggesting different eco-physiological adaptations (Tonolla *et al.* 2000).

Similar to aggregates of small-celled purple sulfur bacteria and *D. thiozymogenes* observed in the chemocline of Lake Cadagno, isolates of both associate in aggregates and display concomitant growth enhancement under conditions promoting sulfur disproportionation in mixed culture even in the absence of the abiotic sulfide scavenger FeOOH (Peduzzi *et al.* 2003). Since the small-celled phototrophic sulfur bacteria *L. purpureus* and *L. roseopersicina* photo-oxidize sulfide to sulfur and further to sulfate (Eichler & Pfennig 1988; Imhoff 2001), small-celled phototrophic sulfur bacteria in the chemocline of Lake Cadagno might therefore act as sulfide scavengers, creating a sink for sulfide produced by sulfur disproportionation of associated sulfate-reducing bacteria (Tonolla *et al.* 2000). The interaction might even be mutualistic, since either sulfate-reduction or disproportionation in association with aggregates of small-celled phototrophic sulfur bacteria might overcome sulfide limitations of small-celled phototrophic sulfur bacteria during periods of intensive photo-oxidation, a situation encountered in Lake Mahoney (Overmann *et al.* 1991, 1994; Overmann 1997) as well as in Lake Cadagno (Joss *et al.* 1994; Fritz & Bachofen 2000; Lüthy *et al.* 2000).

In addition to sulfide, members of the genus *Lamprocystis* can also photo-oxidize elemental sulfur and thiosulfate (Imhoff 2001). During photo-oxidation, globules of elemental sulfur are stored intracellularly as intermediary oxidation products that can be further oxidized or be reduced in the dark by oxidation of internal storage products like glycogen (Mas & van Gernerden 1995). Under such conditions, an association with sulfate-reducing bacteria would be commensalistic since it would not provide an obvious advantage for the purple sulfur bacteria. However, since both organisms are metabolically highly versatile, interactions may not be limited to sulfur compounds only. During sulfate reduction and concomitant oxidation of organic substrates, small organic molecules such as acetate might be excreted by the sulfate-reducing bacteria which could further be used by small-celled purple sulfur bacteria growing under mixotrophic conditions (Eichler & Pfennig 1988).

Intermediate inorganic sulfur compounds such as elemental sulfur, thiosulfate and sulfite are primary substrates for disproportionation. It is still unclear whether these compounds are available to bacterial aggregates in the chemocline of Lake Cadagno, even though deposition of elemental sulfur in the benthic boundary layer potentially supports their availability (Lehmann & Bachofen 1999). For sulfate-rich environments other than Lake Cadagno, higher concentrations of elemental sulfur and polysulfides are generally reported compared to sulfite and thiosulfate concentrations (van Gernerden & Mas 1995). Polysulfides are formed in an abiotic reaction of elemental sulfur with sulfide, and are in a chemical equilibrium with them (Overmann *et al.* 1996) with an oxidation state between sulfide and elemental sulfur (Brune 1988). Polysulfides are electron-donating substrates for anoxygenic photosynthesis by *Thiocapsa roseopersicina*, *C. vinosum* and other purple sulfur bacteria (Brune 1995; Steudel *et al.* 1990; Visscher *et al.* 1990) and can serve as electron acceptors for sulfate-reducing bacteria (Overmann *et al.* 1996; van den Ende *et al.* 1997). In the Lake Cadagno chemocline aggregates, intermediate inorganic sulfur compounds could theoretically be excreted by either partner inside the aggregate and thus have an endogenous origin. However, release of inorganic sulfur compounds other than sulfate by purple sulfur bacteria has not yet been reported (Mas & van Gernerden 1995).

In contrast to green sulfur bacteria, purple sulfur bacteria store elemental sulfur intracellularly, preventing it from direct use by sulfate-reducing partners in the aggregate (Pfennig & Trüper 1992; Mas & van Gernerden 1995), or from reacting with external sulfide to form polysulfides. However, Steudel *et al.* (1990) reported that a minor fraction of sulfide (7%) can be transiently oxidized to thiosulfate by purple sulfur bacteria, and recent findings based on  $\delta^{34}\text{S}$  determinations in Lake Mahoney, indicate that intracellular sulfur of *L. purpurea* reacts with dissolved sulfide to form polysulfides (Overmann *et al.* 1996). In another study, Overmann *et al.* (1997) showed polysulfides and intracellular sulfur constituted significant intermediates in the sulfur cycle of Lake Mahoney. A decrease of polysulfides during summer was accompanied by an increase in numbers of sulfur-reducing bacteria and *L. purpurea* (Overmann *et al.* 1996). These results provide a basis for further studies on the interaction between purple sulfur bacteria and sulfate-reducing bacteria related to *D. thiozymogenes* associated in aggregates in the chemocline of Lake Cadagno. Unfortunately, data on intermediate inorganic sulfur compounds in the chemocline of Lake Cadagno are scarce or not available; a situation that might be due to the fact that these compounds can be consumed at the aggregate level as soon as they are formed either by the small-celled phototrophic sulfur bacteria or the sulfate-reducing bacteria related to *D. thiozymogenes* and thus not be detectable in free water.

It is evident that the role of both sulfur and carbon compounds in the interaction of the bacteria in this association needs to be elucidated in more detail. Since two Lake Cadagno chemocline isolates involved in aggregate formation and association are available, further studies need to address the effects of varying environmental conditions on growth, aggregate formation and association of both organisms. These organisms can certainly be used as model organisms for further in depth studies of the source-sink hypothesis. Future perspectives should also consider other facets of the potential interactions in aggregates, since both types of organisms are highly versatile metabolically, and their interaction may not be limited to sulfur compounds. In addition, the remaining three populations of uncultured small-celled purple sulfur bacteria must be incorporated into these studies, and these should also include an attempt to imitate and maintain the environmental conditions found in the upper part of the chemocline of Lake Cadagno.

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