Development of a real-time PCR method for the detection of fossil 16S rDNA fragments of phototrophic sulfur bacteria in the sediments of Lake Cadagno

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ABSTRACT

Lake Cadagno is a crenogenic meromictic lake situated in the southern range of the Swiss Alps characterized by a compact chemocline that has been the object of many ecological studies. The population dynamics of phototrophic sulfur bacteria in the chemocline has been monitored since 1994 with molecular methods such as 16S rRNA gene clone library analysis. To reconstruct paleo-microbial community dynamics, we developed a quantitative real-time PCR methodology for specific detection of 16S rRNA gene sequences of purple and green sulfur bacteria populations from sediment samples. We detected fossil 16S rDNA of nine populations of phototrophic sulfur bacteria down to 9-m sediment depth, corresponding to about 9500 years of the lake’s biogeological history. These results provide the first evidence for the presence of 16S rDNA of anoxygenic phototrophic bacteria in Holocene sediments of an alpine meromictic lake and indicate that the water column stratification and the bacterial plume were already present in Lake Cadagno thousands of years ago. The finding of Chlorobium clathratiforme remains in all the samples analyzed shows that this population, identified in the water column only in 2001, was already a part of the lake’s biota in the past.

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INTRODUCTION

To predict how organisms interact and adapt to environmental alterations, it is essential to understand the ecosystem responses to change over long timescales. The geological record offers an invaluable opportunity to investigate the variations in biological systems over long periods of time. Molecular approaches have recently been successfully employed to analyze ancient DNA (i.e. fossil DNA) preserved in sedimentary records, such as lake sediments, to reconstruct past ecosystems (Coolen & Overmann, 1998; Coolen et al., 2004a,b, 2008; Bissett et al., 2005; D’Andrea et al., 2006; Coolen & Gibson, 2009; Epp et al., 2010). Permanently stratified lakes especially provide excellent preservation conditions for ancient DNA because of their anoxic bottom waters and undisturbed laminated sediments (Coolen & Gibson, 2009). So far, intact DNA of anoxygenic phototrophic bacteria could be extracted from up to 9100-year-old Holocene lake sediments (Mahoney Lake, Canada) and was analyzed by polymerase chain reaction (PCR) amplification and sequencing (Coolen & Overmann, 1998).

Lake Cadagno, in the southern Swiss Alps (46°33’N, 8°43’E), has an estimated age of 10 000–12 000 years and is the result of glacial erosion during the last glacial period and the formation of a moraine acting as a small dam (Krige, 1918; Boucherle & Züllig, 1988; Stapfer, 1991; Del Don et al., 2001). It represents a unique opportunity for the study of past ecosystems in permanently stratified (i.e. meromictic) lakes in the Alps, as only very few lakes presenting the characteristic stratification remain in this mountain range. The permanent stratification of Lake Cadagno is induced by the presence of underwater saline springs that create a density difference between surface (mixolimnion) and bottom (monimolimnion) waters (Hanselmann & Hutter, 1998; Peduzzi
et al., 1998; Tonolla et al., 1999; Del Don et al., 2001; Tonolla & Peduzzi, 2006). At the interface between the two water layers, a zone of steep gradients of oxygen, sulfide, and light, namely the chemocline, supports the development of a dense microbial community, mainly composed of anoxygenic phototrophic bacteria. The bacteria in the chemocline have been identified using molecular methods, such as 16S rRNA gene clone library analysis and subsequent in situ hybridization with specific fluorescent probes, and belong mainly to two families: the anoxygenic phototrophic purple (Chromatiaceae) and green (Chlorobiaceae) sulfur bacteria (Tonolla et al., 1999, 2003, 2004; Peduzzi et al., 2003a,b).

The aims of the present study were (i) to develop a quantitative real-time PCR methodology for specific detection of 16S rRNA gene sequences of Chromatiaceae and Chlorobiaceae populations in the sediment layers of Lake Cadagno; (ii) to perform a quantitative relative comparison of microbial communities in order to reconstruct past population dynamics and environmental conditions, in particular the presence of meromixis; and (iii) to ascertain whether the predominance of Chlorobium clathratiforme was a previous condition of the lake or is a distinctive trait of the present state.

**METHODS**

**Design of primers and TaqMan probes**

Primers and oligonucleotide probes (Microsynth AG, Balgach, Switzerland) (Table 1) were designed using MEGA (v. 4.1) software (available online: http://www.megasoftware.net/) based on reference probes previously used for in situ hybridization (Tonolla et al., 1999, 2003, 2005; Peduzzi et al., 2011) and modified for real-time PCR. The primers targeted two groups of phototrophic sulfur bacteria that are presently found in the lake’s chemocline: purple sulfur bacteria from the Chromatiaceae family (i.e. Chromatium okenii, Lamprocystis purpurea, Lamprocystis rosseoperina, Lamprocystis sp. population D, Candidatus ‘Thiodictyon syntrophicum’ population F, Thiodictyon chemoclinalis, and Thiocystis cadagnonensis) and green sulfur bacteria from the Chlorobiaceae family (i.e. C. clathratiforme and Chlorobium phaseobacteroides). Thiocystis chemoclinalis and T. cadagnonensis have recently been recognized as species (Peduzzi et al., 2011) as well as Thiodictyon sp. population F described as Candidatus ‘Thiodictyon syntrophicum’ sp. nov. (Peduzzi et al., 2012). Partial (98-bp-long on average) bacterial 16S rRNA gene sequences were selectively amplified using population-specific forward primers (Table 1). TaqMan probes and reverse primers were group specific (Table 1). The internal oligonucleotide probes were labeled at the 5’-end with the fluorophore dye 6-carboxy-fluorescein (FAM) and at the 3’-end with the quencher dye 6-carboxy-tetramethyl-rhodamine (TAMRA) (Microsynth AG). Specificity of primers and probes was checked in silico by comparing the sequences with those in GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Real-time quantitative PCR**

The real-time PCR amplification reactions were conducted using a 7500 Fast Real-time PCR System instrument (Applied Biosystems, Rotkreuz, Switzerland). Optimal conditions were 2 min at 50 °C, 10 min at 95 °C followed by 50 cycles including denaturing (15 s at 95 °C) and primer annealing (1 min at 60 °C). The 20-μL reactions contained 10 μL of TaqMan® Environmental Master Mix 2.0 (Applied Biosystems), 0.9 μM of forward and reverse primers, 0.2 μM of probe, and 2 μL of DNA template. The lack of inhibition was checked by adding a TaqMan® Exogenous Internal Positive Control – VIC™ Probe (Applied Biosystems) to each reaction tube. In addition, each amplification run comprised one reaction devoid of DNA template as a negative control. Finally, quantification of fossil bacterial DNA was performed in duplicates to test reproducibility of the results. The data were analyzed with the 7500 Fast System SDS Software v. 1.4.0 (Applied Biosystems) with the auto settings used for the baseline.
The threshold was set at 0.02. PCR efficacies were calculated (threshold cycle) as a function of the amplicons concentration. The detection limit of the assay was defined as the lowest number of copies that could be detected. Between $10^2$ and $10^6$ copies $\mu$L$^{-1}$ (tenfold dilution series) of specific 16S rDNA amplicons were added to reaction mixtures and served as standards during real-time PCR to calibrate the copy numbers of template DNA in the sediment samples.

Verification of real-time PCR inclusivity and exclusivity

Real-time PCR inclusivity and exclusivity were verified using pure cultures of the nine bacterial populations included in this study. For each culture, we performed a real-time PCR using the population-specific primers-probe set, as well as the primers-probe sets specific to the other eight populations. Additional tests were performed on a control sediment sample free of targeted bacteria obtained from Lake Maggiore, Ticino. The sediment was first dried at 60 °C overnight. Five-gram samples of the control sediment were inoculated with 10 $\mu$L of DNA extracted from the pure culture of all nine study populations. The samples were then submitted to DNA extraction with PowerMax™ Soil DNA Isolation Kit (Mobio, Carlsbad, CA, USA) following the recommendations of the manufacturer. The DNA extracted from the subsamples was then subjected to real-time PCR with the primers and probes for the study populations.

Table 1 Primers and probes used for the real-time PCR

<table>
<thead>
<tr>
<th>Primers/probes</th>
<th>Target*</th>
<th>Oligonucleotide sequence (5¢→3¢)</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProbePSBbis(f)</td>
<td>Chromatiaceae, 16S rDNA, pos. 443–468</td>
<td>CACAAAGAAGACCCGGCTAACCTCGG</td>
<td>This study (consensus)</td>
</tr>
<tr>
<td>S453Cbris(f)</td>
<td>Chromatium okenii (DSM 169), 16S rDNA, pos. 431–453</td>
<td>AACCTGGTGTTATACCTCCATCG</td>
<td>Tonolla et al. (1999)</td>
</tr>
<tr>
<td>S453Abis6(f)</td>
<td>Lamprocystis purpurea (DSM 4197), 16S rDNA, pos. 438–463</td>
<td>GTGCTTGGGAAATACCCGGCGGA</td>
<td>Tonolla et al. (1999)</td>
</tr>
<tr>
<td>S453Bbris(f)</td>
<td>Lamprocystis roseopersicina (DSM 229), 16S rDNA, pos. 433–458</td>
<td>GCATTCTTGGTAAATACCCGGGAAT</td>
<td>Tonolla et al. (1999)</td>
</tr>
<tr>
<td>S453Fbis2(f)</td>
<td>Candidatus ‘Thiodictyon syntrophicum’ (clone 371), 16S rDNA, pos. 434–458</td>
<td>CCCTTGCGYTATACCCATTGAGGG</td>
<td>Peduzzi et al. (2012)</td>
</tr>
<tr>
<td>S453Hbis2(f)</td>
<td>Thiocystis chemochnialis (clone 222), 16S rDNA, pos. 436–456</td>
<td>GGGCCTTAATACCCGTCCGTC</td>
<td>Peduzzi et al. (2011)</td>
</tr>
<tr>
<td>S448bis3(f)</td>
<td>Thiocystis cadagronensis (clone 249), 16S rDNA, pos. 436–454</td>
<td>GCCGGGCCTAATACCCGTCCG</td>
<td>Peduzzi et al. (2011)</td>
</tr>
<tr>
<td>PS8(r)</td>
<td>Chromatiaceae, 16S rDNA, pos. 483–502</td>
<td>GCCGGGCCTAATACCCGTCCG</td>
<td>Peduzzi et al. (2011)</td>
</tr>
<tr>
<td>ProbeGSBbis(f)</td>
<td>Chlorobium clathratiforme (DSM 5477), 16S rDNA, pos. 156–181</td>
<td>GGGCCTTAATACCCGTCCG</td>
<td>Peduzzi et al. (2011)</td>
</tr>
<tr>
<td>Pld190bis(f)</td>
<td>C. clathratiforme (DSM 5477), 16S rDNA, pos. 125–145</td>
<td>ACAATTCGATTGTGCTCCG</td>
<td>Tonolla et al. (2005)</td>
</tr>
<tr>
<td>GS8bis(r)</td>
<td>C. clathratiforme (DSM 5477), 16S rDNA, pos. 220–239</td>
<td>TCCTGCAGCGCTACCGGT</td>
<td>This study</td>
</tr>
<tr>
<td>ProbeGSbis2(f)</td>
<td>Chlorobium phaeobacteroides, (DSM 266), 16S rDNA, pos. 398–421</td>
<td>CGGGACTGACGGTACTCCGCAAT</td>
<td>Tonolla et al. (2003)</td>
</tr>
<tr>
<td>Chlp441bis(f)</td>
<td>C. phaeobacteroides, (DSM 266), 16S rDNA, pos. 373–395</td>
<td>GTGAGAGAAGAATTACCGAGTT</td>
<td>Tonolla et al. (2003)</td>
</tr>
<tr>
<td>GS8bis3(f)</td>
<td>C. phaeobacteroides, (DSM 266), 16S rDNA, pos. 510–535</td>
<td>GAGTTAACCCCCGCTATTGACCC</td>
<td>Tonolla et al. (2003)</td>
</tr>
</tbody>
</table>

f, forward; r, reverse.

*pos., position on the 16S rDNA according to the Escherichia coli numbering (Brosius et al., 1981).

Standard curves and detection limits determination

Total DNA from pure culture cells of the nine targeted bacterial populations was extracted using the InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer’s protocol. The DNA extracted from each bacterial culture was then subjected to PCR by using the primers previously described (Table 1) to amplify the targeted 16S rDNA fragments. The amplicons were purified by gel filtration using Sephadex® G-100 (Sigma-Aldrich, St. Louis, MO, USA) and quantified with the fluorescent dye PicoGreen® (Molecular Probes, Eugene, OR, USA). Serial tenfold dilutions of purified amplicons ranging from $1 \times 10^7$ to $1 \times 10^9$ copies $\mu$L$^{-1}$ were prepared in TE buffer solution. A quantitative standard curve was plotted with the Ct values (threshold cycle) as a function of the amplicons concentration. The threshold was set at 0.02. PCR efficacies were calculated with the formula $[(10^{-1/slope})-1] \times 100$ (Cordier et al., 2007). The detection limit of the assay was defined as the lowest number of copies that could be detected.

The European standard ISO 16140 defines qPCR specificity as target detection without interference of sample components. To verify qPCR specificity, we initially quantified the number of cells contained in 50 $\mu$L of a pure culture of Candidatus ‘Thiodictyon syntrophicum’. Ten microliters of a pure culture of 'Thiodictyon syntrophicum’ without sediment; (iii) 5 g sediment inoculated with 10 $\mu$L of solution containing 1.18 $\times 10^6$ cells of Candidatus ‘Thiodictyon syntrophicum’ (corresponding to 2.36 $\times 10^5$ cells g$^{-1}$); (ii) 50 $\mu$L of solution containing the same amount of cells of Candidatus ‘Thiodictyon syntrophicum’ without sediment; (iii) 5 g sediment inoculated with 10 $\mu$L of solution containing 0.5 $\times 10^6$ copies $\mu$L$^{-1}$ from Candidatus ‘Thiodictyon syntrophicum’, corresponding to a final concentration of $1 \times 10^6$ copies g$^{-1}$ of sediment. For the extraction tests (i) and (ii), a DNase treatment step was performed on the cell suspension before sediment inoculation to eliminate free DNA. Briefly, 5 $\mu$L
Total DNA was extracted from 5 g of wet weight sample using the PowerMax™ Soil DNA Isolation Kit (Mobio). Precautions against contamination of the samples with foreign DNA were applied following Coolen & Overmann (1998). DNA was diluted with 50 μL TE buffer solution and the concentration of extracted DNA was quantified with a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To standardize the results, dry weight was calculated for each sample by allowing 1 g of wet sample to dry overnight at 60 °C. The percentage of humidity was calculated as (wet weight−dry weight)/wet weight × 100. The dry weight of the 5-g sediment sample was then extrapolated. For each of the sediment core samples, the number of copies of 16S rDNA of the nine targeted bacterial populations was quantified by real-time PCR. The results obtained by real-time PCR were normalized to copies μg−1 of extracted DNA to compensate for possible variation in the extraction efficiencies between core samples.

**Sediment core retrieval and age model**

Two composite cores (inner diameter, 5.9 cm) were retrieved in September 2009 at the same location in the deepest part of the Cadagno lake basin (i.e. 21-m water depth) using a UWITEC percussion piston corer. The undisturbed water sediment interface was recovered with a gravity short corer. Core 1 was transferred to the ETH, Zürich, for detailed sedimentological analysis and radiocarbon dating from terrestrial organic remains. Core 2 was longitudinally split, opened, photographed, and sampled at the Alpine Biology Center in Piora, immediately after its retrieval. Core 2 was sampled in the uppermost meter in 5-cm sections and below into a total of 85 10-cm-long sample intervals. Precautions were taken to prevent cross-contamination as described by Coolen & Overmann (1998), and samples for DNA extraction were stored in 50-mL tubes at −20 °C. The robust age model of core 1 could be transferred to core 2 on the basis of over 68 tie points established by visual correlation. The radiocarbon ages were calibrated using the online calibration software OxCal 4.1 (https://c14.arch.ox.ac.uk/~oxcal/OxCal.html) (Bronk Ramsey, 2009) and the Intcal09 calibration curve (Reimer et al., 2009). All ages are reported as calibrated years before present (cal yr BP), where 1950 is by convention the origin of the BP scale. It is however important to note that the sedimentary column of Lake Cadagno consists more than half of mass-movement material and flood deposits, which contain no paleoenvironmental information. Therefore, sample intervals containing solely reworked material were excluded from the DNA extraction. Sixteen samples were then chosen for DNA extraction in this study.

**Extraction of total DNA**

Total DNA was extracted from 5 g of wet weight sample using the PowerMax™ Soil DNA Isolation Kit (Mobio). Precautions against contamination of the samples with foreign DNA were applied following Coolen & Overmann (1998). DNA was diluted with 50 μL TE buffer solution and the concentration of extracted DNA was quantified with a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To standardize the results, dry weight was calculated for each sample by allowing 1 g of wet sample to dry overnight at 60 °C. The percentage of humidity was calculated as (wet weight−dry weight)/wet weight × 100. The dry weight of the 5-g sediment sample was then extrapolated. For each of the sediment core samples, the number of copies of 16S rDNA of the nine targeted bacterial populations was quantified by real-time PCR. The results obtained by real-time PCR were normalized to copies μg−1 of extracted DNA to compensate for possible variation in the extraction efficiencies between core samples.

**Test of variance on extraction**

A test of variance on extraction and real-time PCR was conducted using four subsamples of one sediment from Lake Cadagno known to contain fossil DNA of *C. okenii*. Total DNA was extracted from the four subsamples using the PowerMax™ Soil DNA Isolation Kit (Mobio) as described previously. The number of copies (g dry weight sample)−1 of 16S rDNA of *C. okenii* was then quantified by using real-time PCR with the specific primers and probe. Mean number of copies was 6 × 108 copies (g dry weight sample)−1. Variability among the samples was very low (Var = 0.0006). Therefore, one subsample was used for the DNA extraction from sediments.

**RESULTS**

**Standard curves and detection limits**

Standard curves were created for each primers/probe set (for each of the nine targeted bacterial populations) by plotting the C_v values (threshold cycle) as a function of the number of 16S rDNA copies. All standard curves showed a good confidence in correlating the quantity values to C_v values ($R^2 > 0.96$ in all cases). The amplification efficiency was on average 104% (±SD 13.9). The detection limit (i.e. the sensitivity) of the TaqMan real-time PCR varied among the targeted bacterial populations. *Chromatium okenii, Candidatus ‘Thiodictyon syntrophicum’, and C. placobotectorides* showed the highest sensitivity with down to 5 16S rDNA copies μL−1 detected. Most of the populations (i.e. *L. purpurea*, *L. roseopersicina*, *T. chemoclinalis*, and *C. clathratiforme*) showed a limit of 50 16S rDNA copies μL−1 detected. Two populations (i.e. *T. cadagnonensis* and *Lamprocystis* sp. population D) showed a sensitivity of only 500 copies of 16S rDNA μL−1 detected.

**Verification of real-time PCR inclusivity and exclusivity**

To verify real-time PCR inclusivity and exclusivity, tests were performed on the pure cultures of the nine bacterial study populations. Each pure culture was subjected to real-time PCR using the primers–probe set specific to the population as
well as the primers–probe set specific to the other eight populations. In addition, nine control sediments were inoculated each time with 10 µL of DNA extracted from the pure culture of a bacterial population, for all nine study populations. In each case, the amplification of the targeted bacterial population by real-time-PCR-specific primers–probe sets was positive (data not shown), demonstrating the inclusivity of the real-time PCR. The real-time PCR performed with primers–probe sets for the non-targeted bacterial populations did not show amplification (data not shown), demonstrating the exclusivity of the real-time PCR.

**Verification of real-time PCR specificity**

The number of copies recovered by real-time PCR following the extraction from (i) a control sediment inoculated with 2.36 x 10^5 cells g⁻¹ of Candidatus ‘Thiodictyon syntrophicum’ and (ii) a solution containing the same number of cells but without sediment were in the same order of magnitude (3.2 x 10^5 and 4.8 x 10^5 copies g⁻¹ dry weight sample, respectively). These results showed that the presence of sediment did not affect the efficiency of DNA extraction of the bacterial population and the qPCR specificity. The number of copies recovered by real-time PCR from (iii) the control sediment inoculated with 1 x 10^6 copies g⁻¹ of Candidatus ‘Thiodictyon syntrophicum’–specific amplicons, was 8.5 x 10^2 copies g⁻¹ dry weight sample, thus much lower than the number that was originally inoculated in the sediment. This could be due to the much smaller size of amplicons (approximately 100 bp) that we used for this test, compared to whole cell DNA. The small-sized amplicons are possibly more likely to be retained by the sediment during the extraction process. Consequently, only a part of the inoculated amplicons might have been extracted from the sediment and quantified through real-time PCR.

**Total DNA extracted from the sediment core**

After optimization of the extraction protocol, genomic DNA was isolated from the 16 sediment samples from core 2. Between 1 and 126 µg of DNA was extracted per g of dry weight sample (Fig. 1). Total DNA concentration varied among the different sediment depths. In the older part of the sediment core from 9500 to 1120 cal yr BP, total DNA concentration did not exceed 10 µg g⁻¹ dry weight sample (Fig. 1). The amount of DNA gradually increased in the upper sediment core. Between 1100 and 100 cal yr BP, total DNA concentration per g dry weight sample varied between 10 and 40 µg. The highest amount of DNA was recovered in the first 10 cm of the sediment, which corresponds to the most recent century of the lake’s history (Fig. 1).

![Real-time PCR detection of fossil 16S rDNA fragments](image)

**Specific amplification and quantification of 16S rDNA from purple and green sulfur bacteria**

The results obtained by real-time PCR were normalized to copies µg⁻¹ of extracted DNA to compensate for possible variation in the extraction efficiencies between core samples (Fig. 2). The real-time PCR analysis revealed that there was always at least one of the studied species of bacteria present in each sediment sample analyzed, down to a depth of almost 9 m, corresponding to approximately 9450 cal yr BP (Fig. 2). A lower amount of 16S rDNA was recovered in older sediments compared to more recent ones, although variations were present between the populations. For Candidatus ‘Thiodictyon syntrophicum’, 16S rDNA was detected starting from 2535 to 2620 cal yr BP, while for three other populations, namely L. purpurea, T. cadagnonensis, and, to some extent, L. roseopersicina, the fossil 16S rDNA was not detected before 2110–2160 cal yr BP. In contrast, the 16S rDNA of Lamprocystis sp. population D was not found in sediments between 1595 and 2620 cal yr BP, but was present in older sediments as well as in the upper part of the core. For all nine populations of phototrophic bacteria analyzed, the highest values of 16S rDNA were detected in the most recent deposited sediment (Fig. 2). Higher amounts of 16S rDNA were found in the sediment samples younger than 140 cal yr BP compared to the older part of the sediment core. The fossil 16S rDNA of C. okenii and C. phoebacteroides was found in all analyzed samples and showed less variability compared to the other
populations. *Chlorobium clathratiforme* was also recovered from all analyzed sediment samples and showed a clear increase starting from 105 to 140 cal yr BP upwards.

**DISCUSSION**

In this study, we were able to develop a highly specific real-time PCR method for the detection and quantification of nine phototrophic sulfur bacterial populations in sediment samples. This method is based on a TaqMan real-time PCR amplification of partial bacterial 16S rDNA gene sequences (98 bp on average). The real-time PCR was sensitive, although the detection limit varied among the targeted populations. The method was consequently applied to the analysis of the fossil remains of the nine bacterial populations in a sediment core extracted from Lake Cadagno.

We were able to successfully extract fossil DNA from Lake Cadagno’s sediment down to a depth of 9 m. Through amplification of fossil 16S rDNA sequences, we were also able to detect the presence of fossil DNA of phototrophic sulfur bacteria back to approximately 9500 years BP, providing the first evidence for the presence of DNA of anoxygenic
phototrophic bacteria in Holocene sediments of an alpine meromictic lake. It is thus reasonable to conclude that the water stratification (i.e. meromixis) and phototrophic sulfur bacteria have been present in Lake Cadagno since then. The importance of phototrophic sulfur bacteria for the sulfur, carbon, and nitrogen cycles in Lake Cadagno has already been underlined by various studies (i.e. Luthy et al., 2000; Camacho et al., 2001; Halm et al., 2009; respectively). The efficient preservation of fossil DNA in Lake Cadagno’s sediment can be attributed to low temperatures (Willems et al., 1999), anoxic conditions, and a high concentration of organic material (Coolen & Gibson, 2009).

Our study shows a general decrease in the amount of fossil 16S rDNA quantified from sediments older than 200 years (corresponding to a sediment depth of approximately 24 cm, Fig. 1) in all studied populations, suggesting the possibility of DNA degradation after a given time after deposition. According to a study by Birch et al. (1996) on a short sediment core, the content of water and organic matter in the sediments showed a gradual decrease from the subsurface downwards to 50 cm sediment depth, indicative of a slightly increasing compaction and decomposition with depth, and a corresponding decrease in porosity. This is also confirmed by our age model showing a strong decrease in sedimentation rate in the topmost 50 cm. However, while remains of the populations L. purpurea and T. cadagnonensis were not found in sediment samples older than 2200 years (Fig. 2), vestiges of the other five populations were found in the oldest part of the sediment core, thus providing evidence in favor of the idea that in Lake Cadagno, 16S rRNA gene fragments remain preserved in the order of magnitude of thousands of years after its deposition. The extent to which fossil DNA is preserved and the factors that control the preservation of DNA remain largely unknown (Coolen & Gibson, 2009). A study by Coolen & Overmann (1998) in similar settings showed that the majority of the genomic DNA was degraded to shorter fragments (approximately 600 bp) within the first several thousand of years after deposition. The same study also showed that further decrease in fragment size did not occur between 4000 and 10 000 cal yr BP. From the results of the present study, it seems that degradation to smaller than 100-bp-long fragments did not occur. Thus, possible real-time PCR bias owing to degradation was very limited. When quantifying the amount of 16S rRNA gene fragments from sediments, it is however important to take into account other methodological aspects like lysis efficiency in DNA extraction, amplification efficiency, and detection limits of the PCR (Lenk, 2006).

A general higher quantity of fossil 16S rDNA in the core samples <200 years old could possibly be linked to the increased presence of human habitations and agriculture around the lake, followed by anthropogenic emissions in Lake Cadagno and consequent eutrophication (Boucherle & Züllig, 1988). The construction of the dam in 1948 could also have had an impact on the bacterial production and on the deposition of organic matter, which could be reflected in the first few cm of the sediment. However, in contrast to the large increase in pigment concentration that was observed by Boucherle & Züllig (1988), no particular effects on the amount of total DNA in the time period since the construction of the dam were noticed (Fig. 1).

In addition to the successful extraction and amplification of fossil 16S rDNA of phototrophic sulfur bacteria, nine populations of the two groups of purple- and green sulfur bacteria could also be individually distinguished (Fig. 2). Although other studies on the sediments of Lake Cadagno showed the presence of Cladocera (Crustacea) remains, bacterial pigments (Boucherle & Züllig, 1988), and sulfate-reducing bacteria and methanogenic Archaea (Bottinielli, 2008) down to 170-cm sediment depth, this is the first study that proves that specific sulfur bacterial populations have been present since the early Holocene soon after the formation of the lake. Because of the variability in the quantity of population-specific 16S rDNA fragments amplified from various sediment depths, we can speculate that complex population dynamics occurred throughout the existence of Lake Cadagno. The comparison of 16S rDNA quantities between the nine studied bacterial populations showed that at least one population of purple sulfur bacteria was always present in the sedimentary record (Fig. 2). This corresponds to the constant recovery by Züllig (1985) of the pigment okenone, typically found in this group of bacteria, in the first 170 cm of sediment. There did not seem to be a striking dominance of one or more populations over the others (Fig. 2).

We were surprised to recover remains of the green sulfur bacterium C. clathratiforme from the sediments. According to the yearly analyses on the water column carried out since 1994, this population was not present in the lake before 2001 and was thought to be a new incomer (Bosshard et al., 2000a,b; Tonolla et al., 2005; Gregersen et al., 2009). However, the present study demonstrated that the population was already part of the lake’s biota in the past (Fig. 2). The identification of C. clathratiforme from the sediment does not confirm Gregersen et al.’s (2009) hypothesis that the strain might have been recently introduced into the lake through human intervention. Instead, it supports the hypothesis that C. clathratiforme was already present in the lake before 2000 as an undetectable population and that a change in environmental conditions allowed it to bloom (Tonolla et al., 2005; Gregersen et al., 2009).

CONCLUSIONS

This work contributes to the knowledge on the microbial ecology of Lake Cadagno by providing new information for the reconstruction of past population dynamics of phototrophic sulfur bacteria. It was also possible to determine that the green sulfur bacteria C. clathratiforme was already part of the lake’s biota in the past and it is thus not a newcomer.
characterizing the present lake state. Future studies could extend the identification range of fossil bacterial remains by sequencing and subsequent phylogenetic analysis to better understand the small-scale microbial dynamics in this specific lake setting. In addition, sediment cores from different depth zones of the lake could be used to confirm our results and constrain the variability in the lateral extent of the chemocline.

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