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ORIGINAL PAPER

Metabolic and molecular characterization of bacterial community associated to Patagonian Chilean oligotrophic-lakes of quaternary glacial origin

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Abstract The Patagonian Lakes have particular environmental conditions with or without intermittent disturbances. The study of the microorganisms present in aquatic ecosystems has increased notably because they can be used as micro-scale bioindicators of, among others, anthropogenic pollution and climatic change. The aim of the work was to compare the composition of the bacterial communities associated with sediments of three Patagonian Lakes with different geomorphologic patterns and disturbances. The lake sediments were characterized by molecular techniques, physiology profiles and physico-chemical analyses. The metabolic and physiological profiles of the microbial community demonstrated that non-impacted Tranquilo Lake is statistically different to impacted Bertrand and Plomo Lakes. Similar results were detected by DGGE profiles. FISH results demonstrated that betaproteobacteria showed the highest count in the Tranquilo Lake while gammaproteobacteria showed the highest counts in the Bertrand and Plomo Lakes, indicating that their sediments are highly dystrophic. The results demonstrate differences in the metabolic activity and structural and functional composition of bacterial communities of the studied lakes, which have different geomorphological patterns due to disturbances such as volcanic activity and the climatic change.

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Introduction

The formation of the Patagonian Lakes, in Southern Chile, is related to the dynamics of ice flows during the quaternary glacial pulses, with an estimated age of approximately 14,000 years (Harrison et al. 2005; Glasser and Harrison 2005). Lakes on both sides of the Andes are deep oligotrophic monomictic and of glacial origin (Campos 1984; Geller 1992). They are highly transparent (euphotic depth up to 50 m), have low salt and nutrient concentrations, low productivity, low species diversity (Campos et al. 1992; Woelfl 2007) and they are influenced by the strong climatic gradients across southern South America and are, therefore, extremely sensitive to climatic change (Aniya and Enomoto 1986).

The interconnected Bertrand and Plomo Lakes are limited by a terminal moraine (Glasser et al. 2005) and have been under intermittent disturbances caused by volcanic activity and important ice-masses released from the North Patagonian ice-field surrounding the eastern slope slopes of the valley. These slopes are characterized by strong gradients on jointed rocks having abundant silt and clay leaft resulting from the glacio-fluvial drag glacial lake outburst due to moraine dam failure and covered by overripe forests (Glasser et al. 2005, 2006; Huggel 2004; Borgel 1992). In addition, the impact by volcanic activity in the area must be mentioned (Kratzmann et al. 2009; Scasso and Carey 2005; Bennett et al. 2000).

Tranquilo Lake is surrounded by a large ice-free valley which trends west to east and drains one of the

contemporary outlet glaciers of the North ice-field: the Exploradores Glacier (Glasser and Harrison 2005; Glasser et al. 2006). The area is characterized by a landform–sediment association consisting of ice-scoured schist bedrock with well-developed bedrock landforms (whalebacks) interspersed with glacial and glaciofluvial sediments (Glasser and Harrison 2005). Finally the moraines around Tranquilo Lake reflect stages of restricted glaciations confined to high valleys and more extensive valley-based glaciations (Harrison et al. 2004).

Due to its geographical location these oligotrophic moraine-dammed lakes have particular environmental conditions with or without intermittent disturbances, such as shortages of food freezing/thawing and phenomena associated with climate change (Díaz et al. 2000; Rogora et al. 2008). A strong and frequent disturbance will cause the disintegration of the microhabitats and disruption of the boundary between populations, allowing local resources to be available to a greater proportion of the total microbial mass, meaning more individuals but less species (Torsvic et al. 2002). This phenomenon strongly influences the lake biocenosis and improves the presence of several endemic species highly valuable to science (Pardo et al. 2002).

In the last years, the interest of studying the microorganisms present in aquatic ecosystems has been notably increased by their use as bioindicators (Paerl et al. 2003). The utilization of microbiological parameters, as the diversity abundance of species and the metabolic activity allows differentiating aquatic systems because the microorganisms have a high physiological activity and a rapid response to the environmental changes.

The aim of this work was to compare the composition of the bacterial community associated with sediments of Patagonian Lakes of quaternary glacial origin with different geomorphologic patterns and disturbances associated to the climatic change.

Materials and methods

Sampling

Sediment samples were obtained in January 2009, from three Chilean Patagonian Lakes: Bertrand Lake (46°56′31″S 72°51′22″W), Plomo Lake (47°0′20″S 72°56′7″W) and Tranquilo Lake (46°37′49″S 72°47′13″W) (Fig. 1). The lakes are oligotrophic, polymictic with glacial origin. Environmental temperature and precipitation levels do not vary from lake to lake. Sediment cores were extracted using a UWITEC gravity corer, implemented with a Plexiglas tube of 6 cm diameter. In the field, the first 2–3 cm of each core were collected and placed in polyethylene bags, refrigerated at 4°C, and then transported to laboratory for further processing. The coring site was selected using a Garmin 178C echosounder and the water depth was 29 m for Plomo Lake, 22 m for Bertrand Lake and 24 m for Tranquilo Lake.

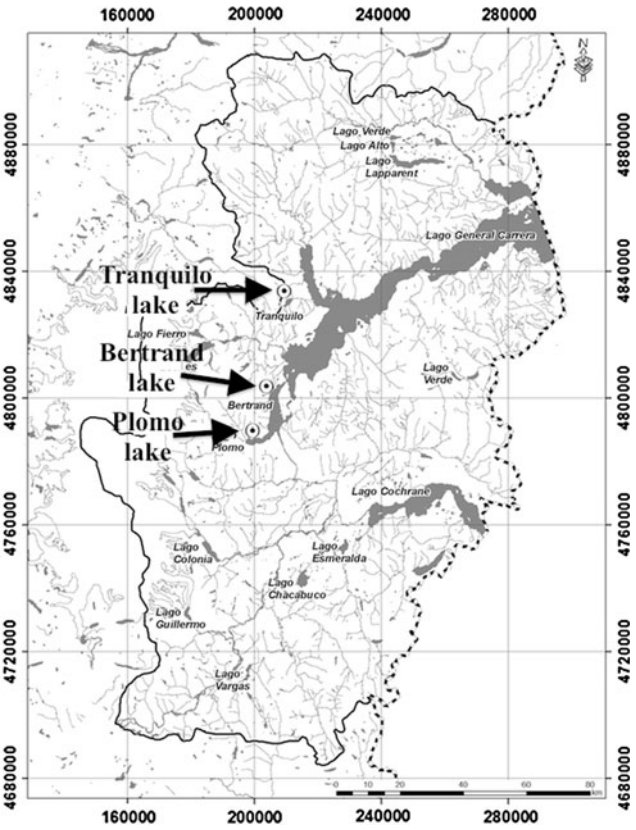

Fig. 1 Sampling sector of three Patagonian Lake Chile. Bertrand Lake (46°56′31″S 72°51′22″W) Plomo Lake (47°0′20”S 72°56′7″W) and Tranquilo Lake (46°37′49″S 72°47′13″W)

For in situ hybridization, sediments samples were fixed for 3 h with paraformaldehyde as previously described (Amann et al. 1995). Samples were stored in a 1:1 mixture of phosphate-buffered saline [130 mM sodium chloride, 10 mM sodium phosphate buffer (pH 7.2)] and 96% ethanol at −20°C. For PCR amplification of the 16S rDNA, unfixed aliquots of sediment samples were frozen at −20°C.

Physico-chemical analyses

Using grain size analysis, samples were sieved at 4.0 and 1.0 φ units and separated into fine (mud) and coarse (sand) fractions. Grain size was analysed using an Elzone 282 PC coulter counter particle analyser. Total organic matter (% TOC) in each core was estimated by the loss on ignition (LOI) technique following the method described by Boyle (2002). Chemical oxygen demand (COD) and biologic oxygen demand (BOD5) were measured according to standard methods (APHA-AWWA-WPCF 1985). Total

nitrogen and phosphorous and nitrogen-like ammonia were measured using Merck Spectroquant kits (Merck KGaA Darmstadt Germany) and quantified in a spectrophotometer (Spectroquant NOVA 60; Merck KGaA). Two replicates were analysed in slices for each lake.

The physico-chemical characteristics of the sediments were analyzed by principal component analysis (PCA) and SIMPER, included in the PRIMER V.6 software package (Clarke and Gorley 2001).

DNA extraction PCR and DGGE

Total DNA from sediment samples was extracted using the UltraClean soil DNA extraction kit (MO BIO Laboratories Inc.) following the protocol provided by the manufacturer. Total DNA of each sample was amplified with 16 s rRNA universal primers EUB 9-27 and EUB 1542 (5′-GAG TTT GAT CCT GGC TCAG-3′) and (5′-AGA AAG GAG GTG ATC CAG CC-3′) (Brosius et al. 1978). Nested PCR was performed using the primer pair 341f and 534r (5′-CCT ACG GGA GGC AGC AG-3) and (5′-ATT ACC GC GGC TGC TGG-3′) with a GC clamp (CGCCC GCCGC GCGCG GCGGG CGGGG CGGGG GCACG GG GGG) (Muyzer et al. 1993) attached to the forward primer. Hot-start PCR was carried out in a 50 µl reaction mixture containing 5 µl of 10× buffer provided by the manufacturer (Sigma) with 15 mmol l^{-1} of $MgCl_2$, 1 µmol l^{-1} of each primer, 200 µmol l^{-1} of deoxynucleoside triphosphates, 1 U of *Taq* DNA polymerase (Sigma) and 0.2 to 1.0 µl of DNA extract. The touchdown temperature program consisted of 6 min at 94°C; 30 cycles of 15 s at 94°C 30 s at the annealing temperature and 2 min and 30 s at 72°C; and a final extension at 72°C for 3 min. During the first 20 cycles, the annealing temperature was decreased by 0.5°C in each cycle from 50 to 40°C. For nested PCR with the primer pair 341f and 534r, the temperature program consisted of 2 min at 94°C and 30 cycles of 1 min at 94°C, 1 min at the annealing temperature and one and a half min at 72°C. The annealing temperature was decreased during the first 20 cycles by 0.5°C in each cycle from 65 to 55°C and a final extension lasting 3 min at 72°C was added. PCR products were checked for concentration, purity and appropriate size by agarose gel electrophoresis and Gel Red nucleic acid staining (Biotium) (Campos et al. 2010).

DGGE was performed with a DGGE 1001 system (C.B.S. Scientific Company Inc.). Fifteen µl of PCR products V3 region were applied directly onto 6% (wt vol^{-1}) polyacrylamide gels in 13 TAE (40 mM Tris 20 mM acetate 1 mM EDTA) with denaturant gradient from 20 to 60% (where 100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at a constant voltage of 200 V at 60°C for 6 h. Later, electrophoresis gels were stained for 20 min with SYBR Gold nucleic acid gel stain (Molecular Probes) as specified by the manufacturer and visualized in a transiluminator (UVP Inc). Bands were excised, re-amplified, purified and sequenced in an ABI3100 genetic analyser using the sequencing kit BigDye v.1.1 (Applied Biosystems). Sequences were identified by BLAST (Campos et al. 2010). Two replicates were analysed for each sediment samples.

Analysis of DGGE profiles

Magnified sections of DGGE gels (six per gel) were photographed with a ChemImager 4000 imaging system (Alpha Innotech) and complete images of each gel were reconstructed with Photoshop software (Adobe). Bands defined as those having an intensity of at least 5% of the most intense band in the sample were scored as present or absent at each position in the gel using the Gel-Pro Analyzer 4.0 software package (Applied Maths). For comparison of banding profiles, a binary matrix was constructed based on the presence (1) or absence (0) of individual bands in each lane. The binary data representing the banding patterns were used to generate a pairwise Dice distance matrix. The distance matrix was used for constructing a multidimensional scaling diagram (MDS) a two-dimensional map with artificial *x*- and *y*-axis where each DGGE fingerprint is placed as one point in a way that similar samples are plotted together. Clustering analysis and MDS were performed with PRIMER V.6 software package (Clarke and Gorley 2001).

DGGE patterns or unique operational taxonomic units (OTUs) were also examined using two indexes in order to scope the multiple aspects of microbial diversity. The Shannon–Weaver index of diversity H (Shannon and Weaver 1963) and the equitability index E (Pielou 1975) were calculated for each sample as follows:

$$H = -\sum (n_i/N)\ \log (n_i/N)$$

$$E = H/\log\ S$$

where n_i is the relative surface intensity of each DGGE band, S is the number of DGGE bands (used to indicate the number of species) and N is the sum of all the surfaces for all bands in a given sample (used as estimates of species abundance) (Fromin et al. 2002). Statistical significance of variance in indexes was evaluated with a one-way analysis of variance (ANOVA). Tukey's post hoc test was selected to separate the means, using Minitab software (Version 15, USA).

The range-weighted richness (Rr) is the total number of bands multiplied by the percentage of denaturing gradient needed to describe th e total diversity of the analyzed sample according to the following formula:

$$Rr = (C^2 - Dg)$$

where C represents the total number of bands in the pattern and Dg the denaturing gradient comprised between the first and the last band of the pattern (Marzorati et al. 2008).

Community-level physiological profiles (CLPP)

The metabolic fingerprints of Patagonian Lake-sediment microbial community were measured using Biolog Eco-Plates (Biolog Inc.) (Insam 1997). The 96-well Biolog EcoPlate comprised three replicate wells of 31 carbon substrates and, for each replicate, a control well without a carbon substrate was included. The substrates were carbohydrates, amines, amino acids, carboxylic acids and polymers. Lake-sediment samples (10 g) were added to 100 ml of distilled water in a 250 ml flask and shaken on a wrist action shaker at 250 rpm for 10 min. Ten-fold serial dilutions were made and 150 µl of the mixture of the 10^{-3} dilution was used to inoculate Biolog ECO plates. Plates were incubated at 25°C and the optical density ($\lambda = 590$ nm) of each well was determined at time 0 and every 24 h thereafter up to 96 h using a microplate reader (BIO-RAD Model 550). The rate of colour development on Biolog EcoPlates was determined by calculating an average well colour development (AWCD) for each plate at each reading (Garland 1996). The AWCD for all carbon sources was calculated as a measure of bacterial functional diversity. The formula used was: AWCD $= \Sigma\ (C - R)/31$ where C is colour production within each well (optical density measurement) and R is the absorbance value of the plates control well. The diversity in colour development for all substrates after 96 h incubation was calculated with the Shannon–Weaver Diversity Index as a measure of community-level physiological profile by using the metabolic fingerprint produced. The formula used was: $H' = -\Sigma\ pi$ ln pi where pi in this case is the proportion of AWCD of a particular substrate to the AWCD of all substrates of a certain lake-sediment studied (Fowler et al. 2006; Harch et al. 1997; Olsson et al. 2005; Yan et al. 2000). In the case of Biolog data, species represents individual substrates and pi is the measure of colour change of the ith substrate relative to the sum of the colour changes of all substrates. As a result, the maximum attainable diversity would be 3.43 when soil microbial community responds equally to the 31 substrates used in the Biolog EcoPlate.

AWCD and H' data obtained from the analyses of the CLPP were analysed using single factor ANOVA with Tukey's test as post hoc analysis using Minitab software (Version 15, USA). In addition, community-level physiological profiles (CLPP) were analyzed by principal component analysis (PCA) SPSS Statistics for Windows® 14.0.1.

Fluorescence in situ hybridisation (FISH) and DAPI staining

The sediments samples (1 g) were fixed for 3 h with paraformaldehyde as described before (Amann et al. 1995). The samples were stored in a 1:1 mixture of phosphate-buffered saline (130 mM sodium chloride, 10 mM sodium phosphate buffer [pH 7.2]) and 96% ethanol at −20°C.

FISH analysis where made in accordance with Amann et al. (1995). Specific probes used were EUB338 (5′-GC TGCCTCCCGTAGGAGT-3′), ALF1B (5′-CGTTCGYTC TGAGCCAG-3′), BET42a (5′-GCCTTCCCACTTCGT TT-3′) and GAM42a (5′-GCCTTCCCACATCGTTT-3′) for Bacteria the alpha, beta and gamma subclasses Proteobacteria, respectively (Manz et al. 1992) and CFB286 for Bacteroidetes (Weller et al. 2000). All samples used for these probes were fixed using the protocol for Gram-negative bacteria with paraformaldehyde, which renders most Gram-positive bacteria unlabelled as previously described (Manz et al. 1992). DAPI-staining (46-diamino-2-phenylindoldihydrochloride-dilactate) was applied after fixation of the sludge with paraformaldehyde by adding DAPI to a final concentration of 1 mg ml^{-1} for 30 min in the last washing step. Bacterial cells were counted using an Olympus Provis AX70 epifluorescence microscope equipped with a SPOT-RT digital camera (Diagnostic Instruments) and ImagePro (Media Cybernetics) software.

Results and discussion

The influence of glacial-quaternary hydrography in southern Chile is manifested in the existence of Patagonian Lakes. These lakes, on both sides of the Andes, are highly transparent (euphotic depth up to 50 m), have low salt and nutrient concentrations, low productivity and low species diversity (Campos et al. 1992; Woelfl 2007).

Patagonian Lakes of southern Chile are characterized by fine sediments with high proportions of silt developed from volcanic-aeolian sediment, covering glacial sediments and quaternary glaciofluvial sediments (McGee et al. 2010; Rojas and Le Roux 2005). In this study, mud texture was classified, according to Wentworth (1922), as "fine silt" and "very fine silt". In addition, muddy textures were classified as silt or clay and also according to particle size. The results of these studies demonstrated that Bertrand and Plomo Lakes were different to the Tranquilo Lake, the first ones being fine silt and the latter very fine silt.

The asymmetry parameter showed a higher variability where Tranquilo and Bertrand Lakes had negative values (−0.82 and −0.16 respectively) whereas Plomo Lake sediment presented a value of 0.41 (Table 1). All sediments were classified as extremely leptokurtic by level of kurtosis.

Table 1 Muddy textures characteristic of three Patagonian Lake

Samples	Media (u)	Selection	Asymmetry	Kurtic	Texture classification[a]
Bertrand Lake	6.83	0.48	−0.16	4.05	Fine slit
Plomo Lake	6.55	0.44	0.41	5.62	Fine slit
Tranquilo Lake	7.29	0.50	−0.82	4.22	Very fine slit

[a] Texture classification was according to reported by Wentworth (1922)

The analysis of % TOC showed that Tranquilo Lake sediment has a higher TOC percentage when compared to Bertrand and Plomo Lakes. This result was similar to that reported by Barra et al. (2001) in sediments samples from Icalma and Lleu–Lleu Lakes (oligotrophics lakes) with maximum values of total organic carbon of 8.40 and 10% respectively. In addition Bertrand et al. (2005) reported total organic carbon values which ranged from 0.6 to 3.6% in sediments of Puyehue oligotrophic lake (Chile 40°S). According to Woelfl et al. (2003), the percentage of total organic matter was the expected for Patagonian Lakes of glacial origin, deep and oligotrophic with waters of high transparency, low productivity and with low levels of dissolved organic carbon and suspended solids (Soto 2002; Soto and Zuñiga 1991).

The PCA of physico-chemical characteristics of the sediments obtained from Bertrand, Plomo and Tranquilo Lakes demonstrated the presence of three groups: Bertrand Lake (M1–M2) Tranquilo Lake (M3–M4) and Plomo Lake (M5–M6). The results obtained by SIMPER analysis showed that Bertrand and Plomo Lakes are less dissimilar with respect to Tranquilo Lake. This difference is due to the NO_3^-N, NH_4^+-N, conductivity, pH and temperature parameters obtained by the PCA analysis (Fig. 2). The conductivity values ranged from 27.3 to 78.4 μS cm^{-1}. These results are similar to those reported by Woelfl et al. (2010) in a warm-monomictic oligotrophic lake of glacial origin (Caburgua Lake, North Patagonia) obtaining low conductivities values between 30 and 35 μS cm^{-1}.

The metabolic profile level of the microbial community in sediments of Patagonia Lakes was investigated by examining the community potential for sole carbon utilization of the 31 different carbon sources found in Biolog Ecoplates (Insam 1997). The microbial community of Tranquilo Lake-sediment metabolized 31 of the 31 test substrates. The number of metabolized substrates decreased to 17 and 14 for the microbial community of Bertrand and Plomo Lakes, respectively. At 96 h, the difference calculated as AWCD (Fig. 3) varied significantly among sampling sites (ANOVA, $P = 0.005$). Post hoc (Tukey's test) analysis for AWCD revealed that the rate at which the substrates were used was significantly higher in Tranquilo Lake-sediment, compared to both Bertrand Lake-sediment ($P = 0.01$) and Plomo Lake-sediment ($P = 0.009$). The

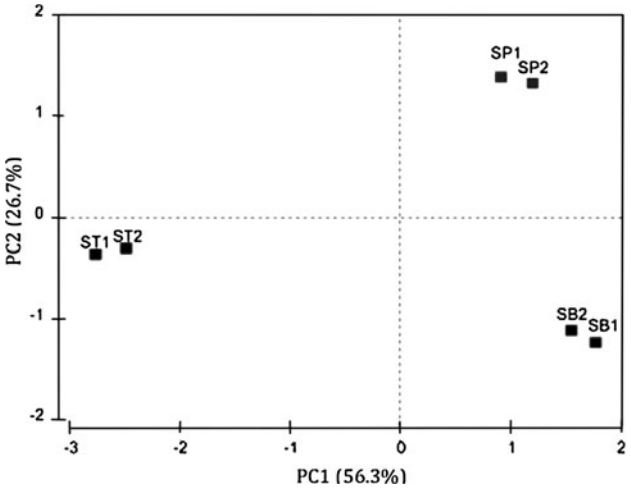

Fig. 2 The principal component analysis (PCA) of physical–chemical characteristics of the sediments obtained from Bertrand (SB) Plomo (SP) and Tranquilo (ST) Lakes

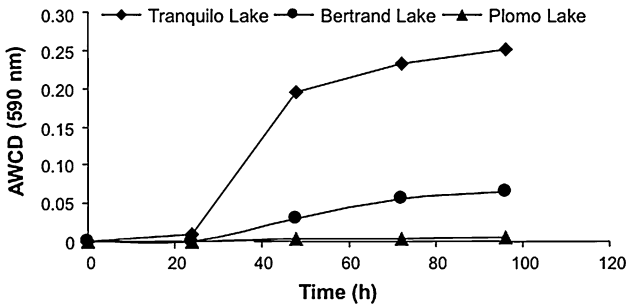

Fig. 3 The average well color development (AWCD) of all carbon sources for the three sediments samples of the Tranquilo Plomo and Bertrand Lakes as a measure of bacterial functional diversity. *Error bars* represent one standard error

Tukey's test showed that Bertrand Lake-sediment was similar to Plomo Lake-sediment ($P = 0.168$). This could be attributed to the fact that Bertrand and Plomo Lakes had been under similar intermittent disturbances. Also, when divided into substrate groups, the average substrate utilization follows the same pattern. Post hoc (Tukey's test) analysis for AWCD revealed that Bertrand and Plomo Lakes are not significantly different for carbohydrates ($P > 0.86$), amino acids ($P > 0.85$), carboxylic acids ($P > 0.61$), polymers ($P > 0.60$) and amines ($P > 0.34$). AWCD values of Tranquilo Lake showed that the sediments

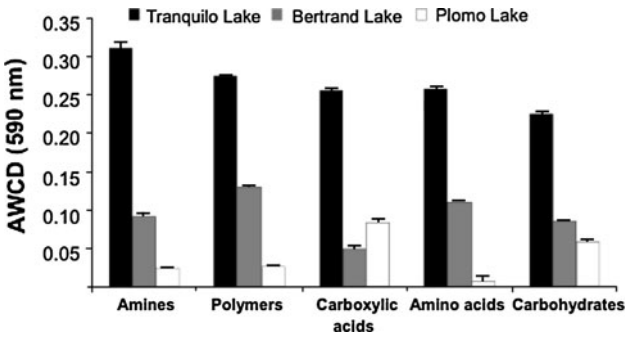

Fig. 4 Relative average well color development (AWCD) calculated from absorbance values of Biolog EcoPlate for the three lake-sediments in study. The absorbance was measure after 96 h incubation. The substrate were divided in five categories; Carbohydrates ($n = 10$) Amines ($n = 2$) amino acids ($n = 6$) Carboxylic acids ($n = 9$) and Polymers ($n = 4$)

of this lake were statistically different from the other two lakes ($P < 0.05$) for all substrate groups (Fig. 4).

The CLPP in Ecoplates was determined using the Shannon–Weaver diversity index (H') (Table 2) and this index varied significantly among sampling sites (ANOVA, $P = 0.004$). Post hoc (Tukey's test) analysis revealed that H' value was significantly higher in the microbial community of Tranquilo Lake-sediment when compared to both Bertrand ($P = 0.026$) and Plomo Lakes-sediment ($P = 0.025$). Bertrand and Plomo microbial communities showed the lowest H' values and Tukey's test showed that they are not significantly different from each other ($P = 0.091$). Lake-sediment microbial diversity of Tranquilo Lake was quite broad at 98.83% of the maximum diversity based in CLPP ($H' = 3.43$). As a result, the Tranquilo Lake-sediment microbial community responds equally to the 31 substrates used in the Biolog Ecoplate. Microbial diversity of Bertrand Lake sample was slightly lower at 81% of maximum diversity. Whereas sediments sample from Plomo Lake had the lowest percentage (52.19%) of the maximum diversity.

Community level physiological profiles indicated differences between lake-sediment microbial functional diversity from the undisturbed site (Tranquilo Lake) and the disturbed sites (Bertrand and Plomo Lakes). The undisturbed site showed a higher average well-colour development (AWCD) when total AWCD was divided into substrate groups and Shannon–Weaver index. In agreement with our results, previous reports also demonstrate a high functional potential of carbon source utilization in microbial communities from non-impacted sites (Gomez et al. 2004).

Principal component analysis was performed. The results showed that the carbon substrate utilizing profiles were clearly separated among the different types of lake sediments samples tested (Fig. 5). In general, sediment samples from Bertrand and Plomo Lakes exhibited a positive PC1 while sediment samples from Tranquilo Lake tended to be negative, suggesting that these samples had a relatively different microbial community function.

Substrate utilization and average metabolic response of microbial communities of Bertrand and Plomo Lakes samples were similar. This can be attributed to their geographical proximity causing that both lakes have similar chemical and physical characteristics, facilitating the settlement of similar species and the same abundance of species.

The bands profile of the bacterial community associated to the three lakes obtained by DGGE shows the presence of 17 bands (Fig. 6). The closest GenBank matches of 16S rDNA sequences obtained by DGGE revealed the presence of 4 bacterial groups, namely alphaproteobacteria, gammaproteobacteria, bacteroidetes and betaproteobacteria, where the gammaproteobacteria and bacteriodetes groups were the most abundant. All the reference bacteria identities were confirmed with 98–100% similarity (Table 3). These groups have been described by other authors in

Table 2 Shannon–Weaver Diversity index (H') of community-level physiological profile (CLPP) in Biolog Ecoplates

Sediment samples	H'	SD
Tranquilo Lake	3.39	0.04
Bertrand Lake	2.76	0.12
Plomo Lake	1.79	0.22

$H' = -\Sigma$ pi ln pi where pi in this case is the proportion of AWCD of a particular substrate to the AWCD of all substrates of a certain sediments use. H' was calculation on values for every single substrate after 96 h incubation

SD standard deviation

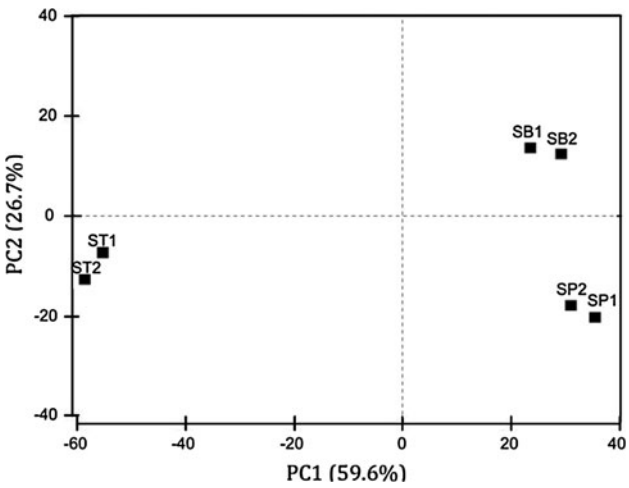

Fig. 5 Principal component analyses of substrates utilizations patterns at three lake-sediments. Bertrand Lake (SB) Plomo Lake (SP) and Tranquilo Lake (ST)

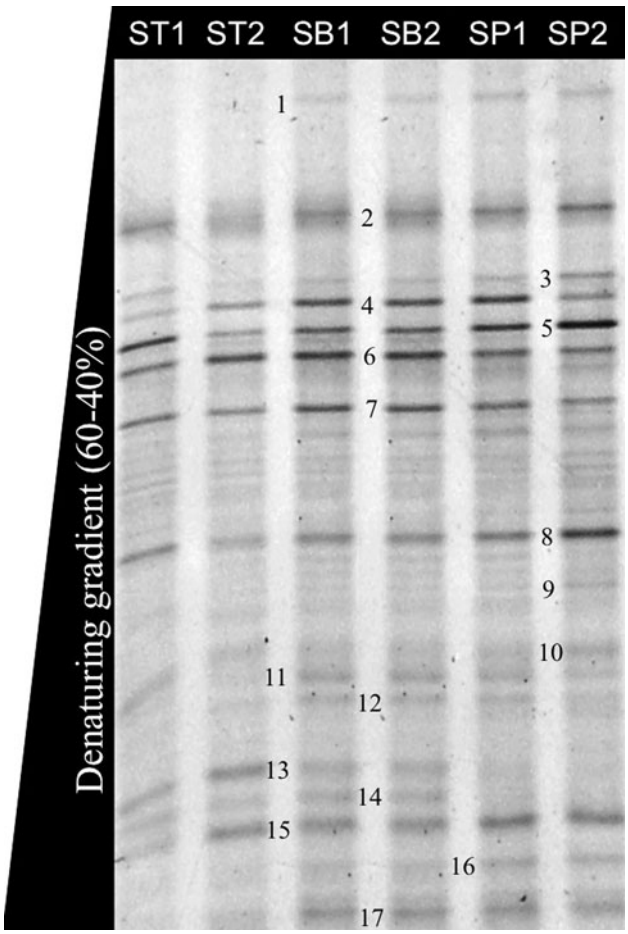

Fig. 6 DGGE of 16S rDNA products amplified with the primers P2 and P3 (GC-clamp). The gel was stained with SYBR-gold and the *bands* indicated in the figure were excised re-amplified purified and sequenced in an ABI3100 genetic analyzer using the sequencing kit BigDye v.1.1 (Applied Biosystems). Tranquilo Lake (ST) Bertrand Lake (SB) and Plomo Lake (SP)

freshwater ecosystems (Cottrell and Kirchman 2000; Imhoff 2006).

In addition DGGE profile bands or OTUs were analysed using the Bray–Curtis correlation. A distance matrix was calculated and a cluster analysis was performed which resulted in a multidimensional scaling (MDS). They showed that the samples obtained from Tranquilo Lake have a low percentage of similarity (40% similarity) when compared to the samples obtained from Bertrand and Plomo Lakes. Bertrand and Plomo Lakes have a similarity of a 70% in the structure of bacterial communities (Fig. 7). These results are alike with the data provided by ANOSIM analysis (global $R = 1$, $P < 0.1\%$), showing significant differences between samples in terms of abundance of OTUs. These results were consistent with the hierarchical cluster analysis (Bray–Curtis index), which clearly indicated the formation of two distinct groups.

The Shannon diversity index (H') such as equity of Pielou (J') and Simpson dominance showed significant differences (ANOVA, $P = 0.006$) between the pattern of OTUs (DGGE profile bands) obtained by 16S rRNA gene analysis by DGGE of Tranquilo, Plomo and Bertrand Lakes (Table 4). Post hoc (Tukey's test) analysis for H' revealed that taking into account the abundance of OTUs there is a greater diversity of species in Tranquilo Lake sediment than in Plomo ($P = 0.001$) and Bertrand Lakes sediments ($P = 0.003$). The Tukey's test showed that Bertrand Lake sediment was similar to that of Plomo Lake sediment in diversity ($P = 0.813$). Moreover the index J' indicates that the abundances are more evenly distributed in the Bertrand Lake than in Tranquilo and Plomo Lakes. Moreover Simpson's dominance index indicates that there is a greater dominance in some OTUs in Tranquilo Lake than in Plomo and Bertrand Lakes. These results are typical of highland lakes because there is low resource availability and extreme weather conditions, allowing the development of a few dominant species (Rautio et al. 2000; Hansson et al. 1993).

The diversity index values of Shannon–Wiener ranged between 1.826 and 1.632. Thus, it is possible to infer that the Patagonian Lakes follow the general pattern observed in lakes located in extreme weather conditions. In addition Patagonian Lakes show low richness and low diversity of species (Rautio et al. 2000; Kilroy et al. 2006). This is based on the fact that bacterial communities in oligotrophic lakes present interesting adaptations to the environment resulting from the peculiar characteristics of these lakes: intense solar radiation, frequent periods of stratification, low amount of gases and ions in solution and low productivity (Capin and Körner 1995; Vila and Muhlhauser 1987).

These results were characteristic of the Patagonian Lakes because the intermittent disturbances such as food shortage, freeze-thawing or climatic change generate altered environmental conditions and the released resources provide new opportunities for species settlement. Thus, the disturbance will ensure that communities include a mix of various stages of succession. However, strong and frequent disturbances will cause the disintegration of the microhabitats and disruption of the boundary between populations, allowing more availability of local resources to a greater proportion of the total microbial biomass, meaning more individuals but fewer species.

Moreover the carrying capacity (Rr) of the bacterial communities from Bertrand and Plomo Lakes reported values lower than 10. These data are attributed to particularly adverse environments or those environments that restrict the settlement and are characterized by low species diversity values (Marzorati et al. 2008).

Table 3 Analysis of 16S rDNA sequences obtained by DGGE

Band	Closest sequence relative	GenBank access no.	Bacterial groups
1	Uncultured bacteroidetes bacterium	GQ870456	Bacteroidetes
2	Uncultured gamma proteobacterium	EF621534	Gammaproteobacteria
3	Uncultured bacteroidetes bacterium	FJ828185	Bacteroidetes
4	Uncultured bacteroidetes bacterium	FJ916551	Bacteroidetes
5	Uncultured bacteroidetes bacterium	HQ442268	Bacteroidetes
6	*Pseudomonas* sp.	AY456703	Gammaproteobacteria
7	Uncultured betaproteobacterium	FR647794	Betaproteobacteria
8	*Sphingomonas* sp.	AB288317	Alphaproteobacteria
10	*Pseudomonas* sp.	HQ824363	Gammaproteobacteria
11	Uncultured beta proteobacterium	EF698301	Betaproteobacteria
13	*Pseudomonas putida*	GU191929	Gammaproteobacteria
14	Uncultured gamma proteobacteria	AF440839	Gammaproteobacteria
15	Uncultured bacteroidetes bacterium	FJ828228	Bacteroidetes
16	Pseudomonadales bacterium	AM931147	Gammaproteobacteria
17	Uncultured bacteroidetes bacterium	GQ870456	Bacteriodetes

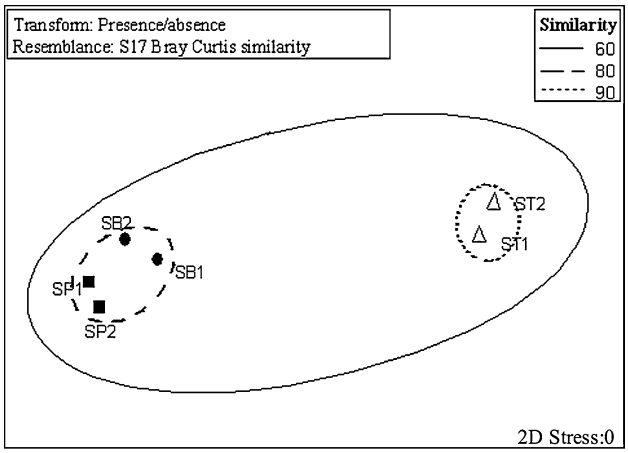

Fig. 7 Multidimensional scaling (MDS) of the DGGE data matrix of Eubacteria rDNA fragments from the Tranquilo Bertrand and Plomo Lake. Similarly index was evaluated for percentage

The variation in the number of OTUs associated with physico-chemical analyses indicates that both characteristics, physical and chemical, of the sediments influence the structure of bacterial communities present in the Patagonian Lakes sediments studied. Zhao et al. (2008) reported, using DGGE, that changes in the bacterial community structure associated with lake sediments could be attributed to changes in physico-chemical conditions of the environment. Similar data were presented by Dorador et al. (2007), analysing lacustrine sediments from the Rapel reservoir (eutrophic system in Chile that has undergone many anthropogenic impacts on recent decades). DGGE and traditional microbiological techniques were used to study the bacterial composition in Rapel Lake. The results showed significant temporal variation in bacterial community concentration and composition in response to changes in physical and chemical composition of water. These results demonstrated that the direct analysis of the 16S rDNA gene dynamics could be used for identifying changes in microbial communities.

The microbial abundance and major phylogenetic groups were examined through DAPI staining and hybridization with specific oligonucleotide probes (EUB 338). The microbial populations and communities were analysed in sediments of the three Patagonian Lakes studied. Total cell numbers were determined by DAPI staining and the number of members of the domain bacteria were counted by hybridization with EUB 338 probe. DAPI results showed that the total cell counts in sediments of Tranquilo, Bertrand and Plomo Lakes were 8.7×10^8,

Table 4 Ecology index obtained from DGGE profiles bands of 16S rDNA analyses

Sample	*N*	*J*′	*H*′	*λ*′
Bertrand	294,646	0.9108	1.632	0.7836
Tranquilo	341,236	0.8781	1.826	0.8225
Plomo	154,419	0.7611	1.672	0.7922

N abundance, *H* Shannon–Weaver, *J* Pielou, *λ*′ Simpson

7.7×10^8 and 5.7×10^8 cells g^{-1}, respectively. Hybridization showed that domain bacteria counts in Tranquilo, Bertrand and Plomo Lakes sediment were 6.5×10^8, 5.3×10^8 and 4.1×10^8 cells g^{-1}, respectively. Due to the fact that 61–74% of the DAPI counts hybridized to the EUB 338 bacterial probe, we assume that domain bacteria were dominant in sediments. It is worth mentioning that Plomo Lake showed the lowest count rate for the domain Bacteria.

Major phylogenetic groups in the sediment cores were investigated. Gammaproteobacteria, bacteroidetes and betaproteobacteria were the groups that showed the highest count of the three sediments studied. Alphaproteobacteria subgroups showed low counts (Fig. 8). In addition, betaproteobacteria and bacteriodetes were the groups showing the highest count in the Tranquilo Lake and gammaproteobacteria and bacteriodetes were the groups showed the highest count in the Bertrand and Plomo Lakes.

Possibly, the high abundance of beta and gammaproteobacteria is linked to the high content of detrital and humic substances in sediment, as these are regarded as a favourite food for proteobacteria. Similar results were reported by Wobus et al. (2003) studying the microbial diversity of sediments from reservoirs of different trophic states, finding that betaproteobacteria constituted an important fraction in the sediments of the more eutrophic reservoirs whereas gammaproteobacteria were most frequently detected in sediment samples from the dystrophic reservoirs. On the other hand, bacteroidetes is a phylogenetically heterogeneous group composed of genera with heterotrophic anaerobic and aerobic metabolism and, together with proteobacteria, are the most abundant groups in both freshwater and marine ecosystems (Cottrell and Kirchman 2000).

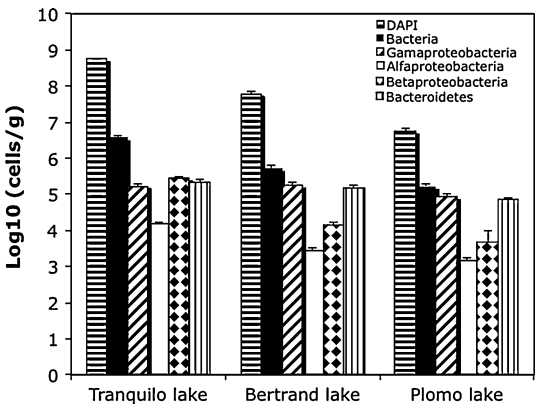

Fig. 8 Bacterial community composition in sediments samples from Tranquilo Bertrand and Plomo Lakes determined by FISH. Probes specific: EUB338 ALF968 BET42a and GAM42a for Bacteria the alpha beta and gamma subclasses Proteobacteria respectively and CFB286 for Bacteroidetes

Conclusion

The results demonstrate differences in the metabolic activity and structural and functional composition of bacterial communities of the studied lakes, which have different geomorphological patterns possibly due climatic change and others disturbances. Therefore the microbial community can be used as an indicator of the state of an ecosystem because it is characterized by a high physiological activity and a rapid response to environmental changes. The diversity of the microbiological communities generally decreases in response to disturbances or environmental stress. The surviving populations have specific properties allowing them to persist in disturbed communities, where different metabolic capabilities can be present. These capabilities might allow that some microorganisms be used as micro-scale sentinels to monitor changes in composition due to anthropogenic pollution, seasonality and spatial distribution.

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