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ENVIRONMENTAL MICROBIOLOGY

Extremophile Culture Collection from Andean Lakes: Extreme Pristine Environments that Host a Wide Diversity of Microorganisms with Tolerance to UV Radiation

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Abstract A total of 88 bacterial strains were isolated from six Andean lakes situated at altitudes ranging from 3,400 to 4,600 m above sea level: L. Aparejos (4,200 m), L. Negra (4,400 m), L. Verde (4,460 m), L. Azul (4,400 m), L. Vilama (4,600 m), and Salina Grande (3,400 m). Salinity ranged from 0.4 to 117 ppm. General diversity was determined by denaturing gradient gel electrophoresis (DGGE) analysis. From the excised DGGE bands, 182 bacterial sequences of good quality were obtained. Gammaproteobacteria and *Cytophaga*/*Flavobacterium*/*Bacteroides* (CFB) were the most abundant phylogenetic groups with 42% and 18% of identified bands, respectively. The isolated strains were identified by sequence analysis. Isolated bacteria were subjected to five different UV-B exposure times: 0.5, 3, 6, 12, and 24 h. Afterwards, growth of each isolate was monitored and resistance was classified according to the growth pattern. A wide interspecific variation among the 88 isolates was observed. Medium and highly resistant strains accounted for 43.2% and 28.4% of the isolates, respectively, and only 28.4% was sensitive. Resistance to solar radiation was equally distributed among the isolates from the different lakes regardless of the salinity of the lakes and pigmentation of isolates. Of the highly resistant isolates, 44.5% belonged to gammaproteobacteria, 33.3% to betaproteobacteria, 40% to alphaproteobacteria, 50% to CFB, and among gram-positive organisms, 33.3% were HGC and 44.5% were Firmicutes. Most resistant strains belonged to genera like *Exiguobaceterium* sp., *Acinetobacter* sp., *Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Sphyngomonas* sp., *Staphylococcus* sp., and *Stenotrophomonas* sp. The current study provides further evidence that gammaproteobacteria are the most abundant and the most UV-B-resistant phylogenetic group in Andean lakes and that UV resistance in bacteria isolated from these environments do not depend on pigmentation and tolerance to salinity.

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Introduction

The Andean Altiplano is a sedimentary–volcanic plateau at an average altitude of 4,000 m, located at latitudes between 8° and 30° south. Several lakes in the Altiplano are formed over evaporitic endorheic basins. Difficult to explore with no access roads, these aquatic ecosystems present arid conditions, resulting in a decrease in the availability of water. Solar irradiance is 165% higher than sea level with instantaneous UV-B flux reaching 17 W m^{-2}. UV flux is twice the amount of present-day equatorial Mars, while UV-B is half the amount on Mars. Low nutrient concentrations, particularly phosphorus; presence of heavy metals, especially arsenic [27]; and large daily air temperature fluctuations, ranging from 20° C during the day to −40°C at night, are other conditions that make these lakes extreme environments for life; they make these lakes similar to early Mars and, consequently, they are interesting for astrobiology studies. Even though the conditions are highly limiting, recent pioneering studies have described the microbial diversity of different Altiplano lakes and revealed unexpectedly diverse microbial communities [8–10].

Considering the high elevation, geography, and physical–chemical characteristics of these wetlands, ultraviolet

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radiation (UVR) is considered one of the most limiting factors for bacterioplankton communities in such ecosystems [16]. According to biological responses, UVR can be divided into three bands: UVR-A, UVR-B, and UVR-C, and high UVR doses are particularly related to cell damage [7]. UVR-B (280–320 nm) is detrimental to life because of the strong absorption of wavelengths below 320 nm by DNA molecules. UVR-A (320–400 nm) causes only indirect damage to DNA, proteins, and lipids through reactive oxygen intermediates [19, 35]. Damage caused by UV in bacterial systems in aquatic environments eventually affects the whole community, having an impact on photosynthesis, biomass production, and the community composition [3, 4, 18, 45]. The effects of UVR on different aquatic systems have been thoroughly studied, especially in marine environments [2, 3, 20, 24, 36]. Studies on the impact of UVR on bacterioplankton have also been carried out in other aquatic systems, such as alpine lakes, measuring the solar UVR incidence on plankton [20, 21, 30, 31, 36–39, 42, 45, 46]. Other authors have done research on biodiversity in the Himalayas [23, 25, 49, 50]. Studies of UV incidences in microbial communities from Andean aquatic systems have been performed in total bacterioplankton communities and in bacteria isolated from surface water. Previous studies at our laboratory have demonstrated that bacteria isolated from different Andean wetlands (Fig. 1) presented high UV-B resistance: *Acinetobacter johnsonii* A2, *Micrococcus* sp. A7, and *Nocardia* sp. A5 from L. Azul (4,400 m), *Pseudomonas* V1 and *Micrococcus* sp. V7 from L. Vilama (4,600 m), and *Pseudomonas* sp. MF10 from L. Pozuelos (3,600 m) [11, 16, 48]. In addition, the impact of solar radiation on bacterioplankton in a hypersaline Andean lake (Laguna Vilama, 4,600 m) was measured in situ, demonstrating that bacterioplankton was well-adapted to high solar irradiance due to the relatively low impact on bacterioplankton diversity [14].

Previous studies have examined the UVR effect on different phylogenetic components of bacterioplankton in diverse environments. Agogue et al. [1] found great interspecific differences among 90 marine isolates with medium and highly UV-resistant strains. Gammaproteobacteria and Bacteroidetes showed to be the most abundant species of the highly UV-resistant strains. Similar results were found for gammaproteobacteria of the Mediterranean Sea [2]. In contrast, only few studies have been carried out in places highly exposed to UVR like high-altitude lakes.

Several hypotheses propose that a broad phylogenetic spectrum of microorganisms, adapted to stress after desiccation or high salinity, can also adapt to radiation stress, since both stress types have deleterious effects on the cells, particularly on DNA, causing a strong oxidative stress [5, 6, 28, 34]. Consequently, tolerance mechanisms to different kinds of stress could be similar in all these environments.

The goals of this paper were (1) to determine if there is any relationship between UV resistance and phylogenetic affiliation and (2) whether bacteria isolated from more saline lakes were more resistant to solar radiation than those isolated from less saline lakes due to common adaptation strategies developed.

To answer these questions, 88 UV-B-resistant strains belonging to different phylotypes isolated from six Andean lakes with different salinities were analyzed: L. Aparejos (4,200 m), L. Negra (4,400 m), L. Verde (4,460 m), L. Azul (4,400 m), L. Vilama (4,600 m), and Salina Grande (3,400 m; Table 1). The general diversity of the total community of the lakes was also determined in order to find out whether isolate filiations were more or less representative for the total community.

Material and Methods

Sampling and Isolation, Cultivation and Identification of the Strains

Samples were collected during austral summer. The first samples were obtained from Salar Laguna Verde, 20 November 2006 and samples from Vilama and Salina Grande were obtained 19 February 2006 and 15 March 2006, respectively. Surface water samples were collected in sterile flasks and stored between 5°C and 10°C until further processing at the laboratory within approximately 24 h.

Some strains from Laguna Azul and Vilama (identified with an A and V in the strain code, respectively) have been described previously [11, 48]. Most isolates from Laguna Aparejos, Negra, Verde, Vilama, and Salina Grande (Ap, N, Ver, V, and SG in the strain code, respectively) are reported in the present paper (Table 3). Bacteria were isolated by plating onto two media: Lake medium (LM) was used to maintain the same salinity as the isolation environment and was obtained by filtering lake water (0.22 μm Biopore filters) and adding 2.5 g yeast extract and 12 g agar (Difco) per liter. MGM medium was used in order to isolate most halophilic bacteria [12]. MGM composition was salt water 30%: 333 mL, tryptone 1 g, yeast extract 1 g (Oxoid), per liter of deionized water. Salt water 30% (pH 7.5): NaCl 240 g, $MgCl_2 \cdot 6H_2O$ 30 g, $MgSO_4 \cdot 7H_2O$ 35 g, KCl 7 gL^{-1}, 5 mL of $CaCl_2 \cdot 2H_2O$ 1 M,Tris–HCl 1 M. Isolated strains were then cultured in the same isolation media at 20°C with shaking. Genomic DNA extractions were carried out as described by Fernández Zenoff et al. [16]. Universal primers were used to amplify and sequence 16S rDNA gene (8-27F–R518 and 8-27F–R1492). Polymerase chain reaction (PCR) products were checked in 0.8% (*w*/*v*)

Table 1 Characteristics of six high-altitude Andean wetlands in Argentina: Laguna Aparejos, L. Negra, L. Verde, L. Azul, L. Vilama, and Salina Grande

Wetland	L. Aparejos	L. Negra	L. Verde	L. Azul	L. Vilama	Salina Grande
Geographic position	Catamarca	Catamarca	Catamarca	Catamarca	Jujuy	Jujuy
Global position	27°34′ S, 68° 23′ W	27°40′S, 68° 23′ W	27°38′ S, 68° 32′ W	27°38′ S, 68° 32′ W	22°35′ S 66° 55′ W	23°36′ S, 66° 55′ W
Depth (cm)	10	20	20	100	20	ND
Altitude (m)	4,200	4,400	4,400	4,400	4,600	3,400
pH	6.5	6.8	6.7	7.5	7.1	ND
Arsenic (mg L^{-1})	2.5	3	0.8	0.8	11.8	ND
Phosphorus (mg L^{-1})	ND[a]	<0.05	<0.012	<0.012	ND[a]	ND
Salinity (ppm)	0.4	32	5	5	117	113
Chlorophyll (μg L^{-1})	6.05	0.63	1.04	0.20	12.80	ND
Max UV-B registered in situ (W m^{-2}; 280–312 nm)	9.80	10.80	10.78	10.78	8.94	ND

ND not determined

[a] Below the detection limit

agarose gels, and DNA sequencing was performed by Macrogen (Korea). The sequences were registered at the GenBank database (Table 3).

Construction of Alignments and Phylogenetic Trees

To construct the phylogenetic trees, the sequences were aligned in the CLUSTALW program and the alignments were manually spot-checked [43]. Analyses were performed by the neighbor-joining method from within the MEGA 4.0 environment [33, 41] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown above the branches [15]. The evolutionary distances were computed using the maximum composite likelihood method [40] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

Figure 1 High-altitude Andean wetland ecosystems in the northern Argentinean Andes. **a** L. Vilama (4,600 m), **b** L. Negra (4,400 m), **c** L. Azul (4,400 m), and **d** L.Verde (4,000 m)

Chlorophyll Salinity and Phosphorus Determination

One hundred milliliters of water sample were filtered through a glass fiber filter (Whatman GF/F). The filters were frozen at −20°C until extraction and then were extracted with 5 mL of methanol 100% at 45°C during 2.5 h in darkness. The suspensions were centrifuged (1,288×g) for 10 min and the absorption spectra were measured between 250 and 750 nm. Chlorophyll values were calculated by the Porra equation [32]. The salinity was determined using a conductivity meter set "Qcond 2400" device, and phosphorus was determined by UV-visible/ 4500 PC spectrophotometry technique.

Irradiation Experiments

UV-B was chosen because this is the most harmful radiation in the high plateau environments. Radiation assays were carried out in duplicate and dark controls were included for all experiments. Bacterial isolates were grown in LM (40 mL) on a laboratory shaker at 25°C and cells were harvested in the early stationary phase by centrifugation (7,000×g for 10 min at 10°C). Then, cultures were washed twice with filtered and sterilized lake water and resuspended in the same volume. The total volume was transferred to sterile 45-mL quartz tubes with a length of 16 cm and a ratio of 0.9 cm. They were placed horizontally to get maximal exposure to radiation. They were irradiated from a distance above 30 cm. Tubes were covered with an acetate sheet to block out UV-C and incubated at 15°C under gentle shaking (25 rpm) and exposed to UV-B (two 09815-06 lamps, Cole Parmer Instrument Company, with major emission line at 312 nm) during 24 h. Irradiance was quantified with a radiometer (09811-56, Cole Parmer Instrument Company) at 312 nm with half bandwidth of 300 to 325 nm. Aliquots of 0.1 mL were extracted after different exposure times (0.5, 3, 6, 12, and 24 h) in order to prepare serial dilutions in LM or MGM. Samples (0.1 mL) of the appropriate serial dilutions were spread in duplicate onto LM or MGM Petri dishes and the number of colony forming units was determined. Controls of unexposed samples were run simultaneously in darkness and the percentage of cell survival after each treatment was calculated relative to these controls. Strains were classified as sensitive (S), medium resistant (R), or highly resistant (R+).

Determination of Different Classes of UVR Resistance

UVR resistance was classified into three categories determining the growth of bacterial isolates following different exposure times (Table 3). Isolates not growing after 30 min of exposure were considered sensitive (S). Isolates growing after 1 h of exposure, but not after 6 h, were considered medium resistant (R) and strains that grew after 6 to 12 h of exposure to UVR were considered highly resistant (R+). Bacteria that survived after 12 h are marked with an asterisk in the corresponding table. An example of different classes of UVR resistance of strains isolated from L. Vilama is shown in Fig. 4c.

PCR Amplification, DGGE, and Sequencing

Water samples (25 mL) were filtered (0.22 μ pore size) and DNA was extracted using the CTAB method [16]. The variable V3 region of 16S rDNA was enzymatically amplified in the PCR with primers to conserved regions of the 16S rRNA genes. The nucleotide sequences of the primers are as follows: primer F357, 5′-TACTGATAGAA TGTGGAGC-3′; R518, 5′-CGT ATT ACC GCG GCT GCT

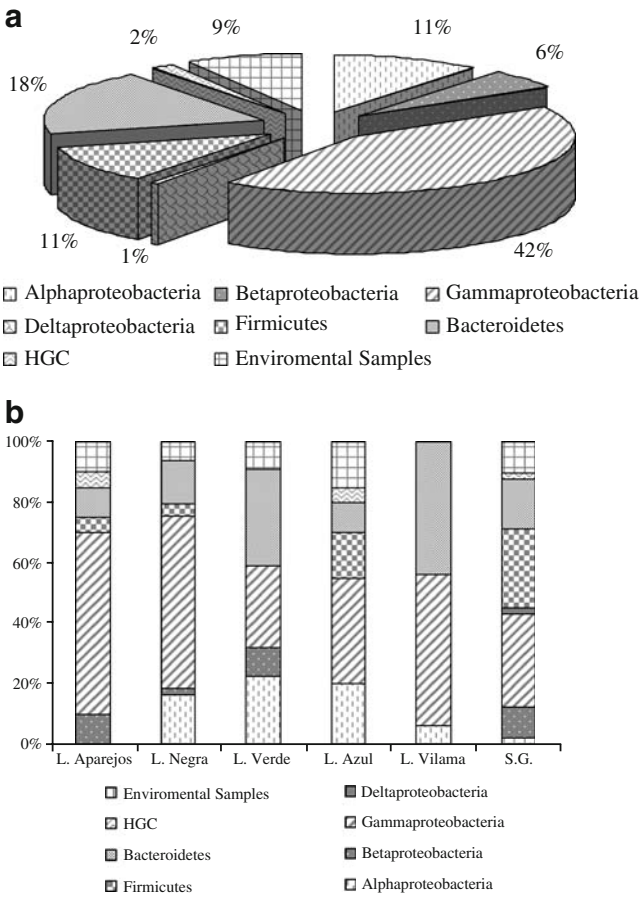
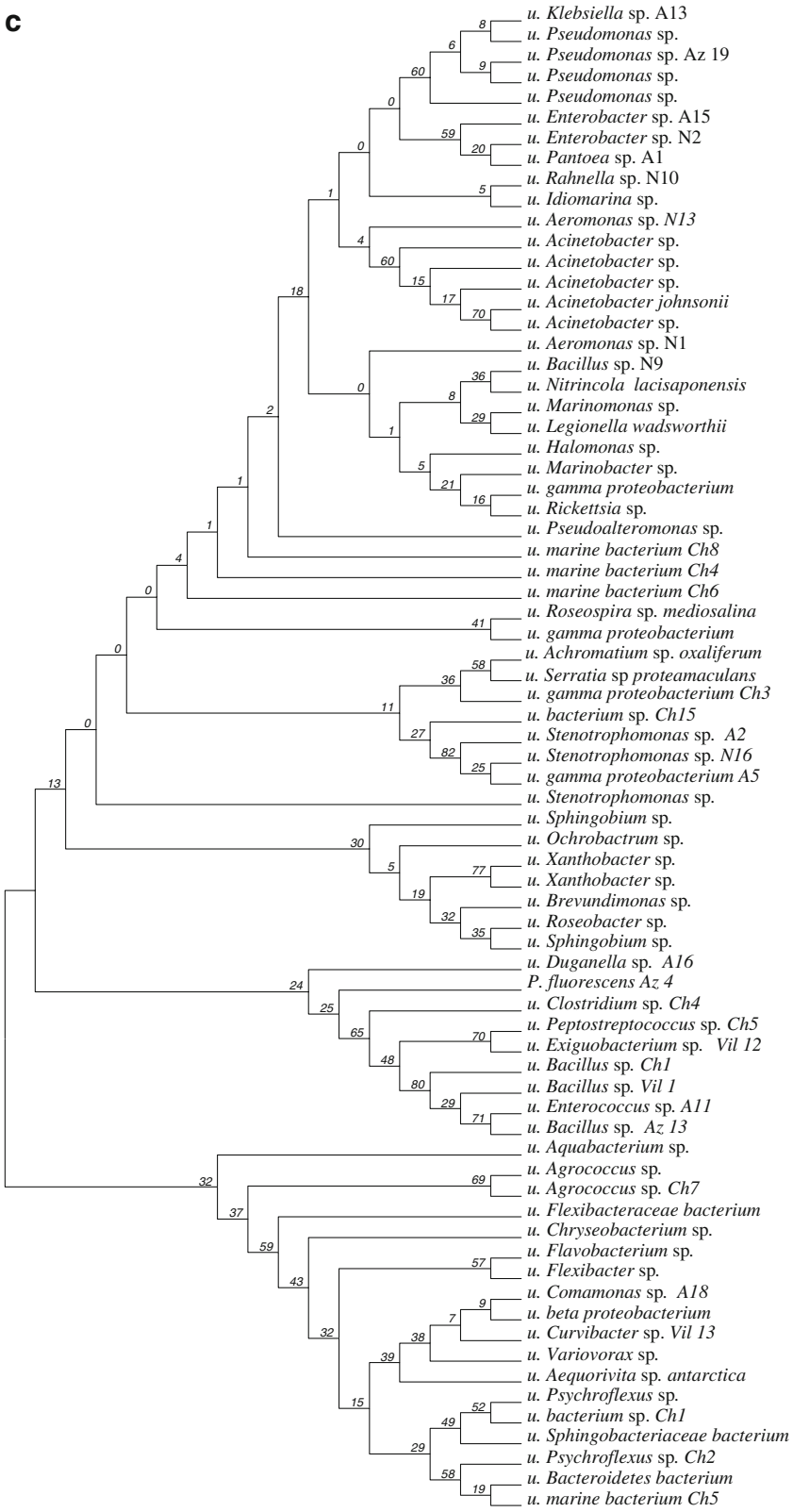


Figure 2 **a** Distribution of DGGE bands among the main phylogenetic groups. **b** Distribution of the main phylogenetic groups of identified DGGE bands among the studied lakes. **c** Neighbor-joining dendrogram based on 16S rRNA gene sequencing showing the phylogenetic position of DGGE bands from all the studied environments. Bootstrap values are shown at the branching points (percentage of 1,000 replicates)

Figure 2 (continued)



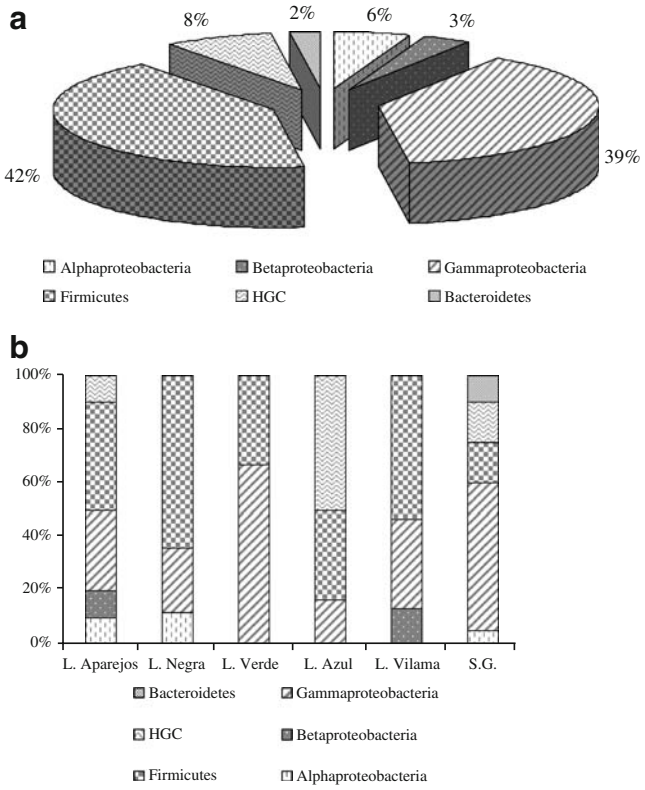


Figure 3 **a** Distribution of extremophile culture collection in the main phylogenetic groups. **b** Distribution of the main phylogenetic groups among the studied environments. **c** Neighbor-joining dendrogram based on 16S rRNA gene sequencing showing the phylogenetic position of isolated strains from all the studied environments. Bootstrap values are shown at the branching points (percentage of 1,000 replicates)

GG-3′; and F341-GC, 5′CGC CCG CCG CGC CCC GCG CCCGTC CCG CCG CCC CCG CCCGCC TAC GGG AGGCAG CAG-3′ [13, 29]. Primer F341-GC contains the same sequence as primer F357 but has at its 5′ end an additional 40-nucleotide GC-rich sequence (GC clamp). A combination of primers F341-GC and 518R or primers 357F and 518R was used to amplify the 16S rDNA regions in the different bacterial species which correspond to positions 341 to 534 in *Escherichia coli*.

PCR amplification was performed with a Biometra Thermocycler using 100 ng of purified genomic DNA, 20 pmol of each primer (Genbiotech), and 200 μmol of each deoxyribonucleoside triphosphate. Five microliters of 10x PCR buffer ($MgCl_2$) and 0.25 U of Go Taq (Promega) were added to a 0.5-mL volume test tube, which was filled up to a volume of 25 μL with sterile Milli-Q-water. Samples were first incubated at 94°C for 5 min to denature the template DNA. Next, the temperature was subsequently lowered to 65°C for 1 min and then decreased by 1°C every second cycle until 55°C. At this temperature, five additional cycles were carried out. PCR extension was performed at 72°C for 3 min and amplification products were analyzed using agarose (1.5% *w*/*v*) gel electrophoresis.

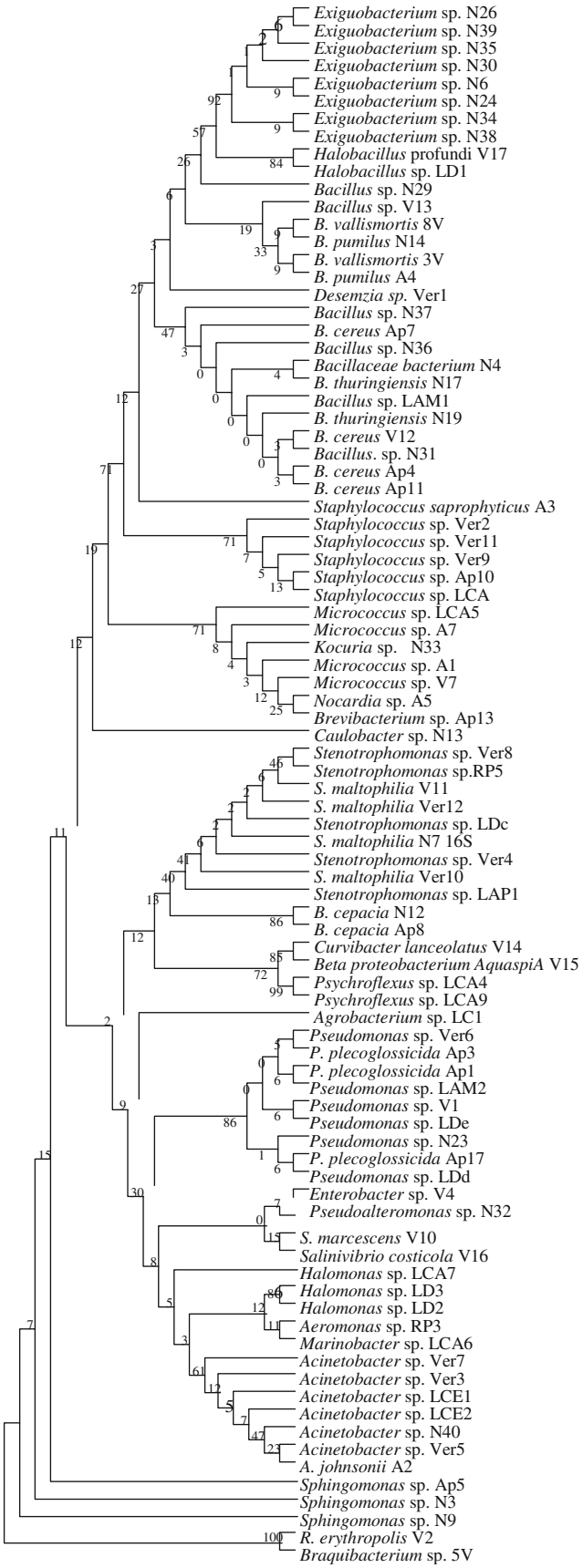


Figure 3 (continued)

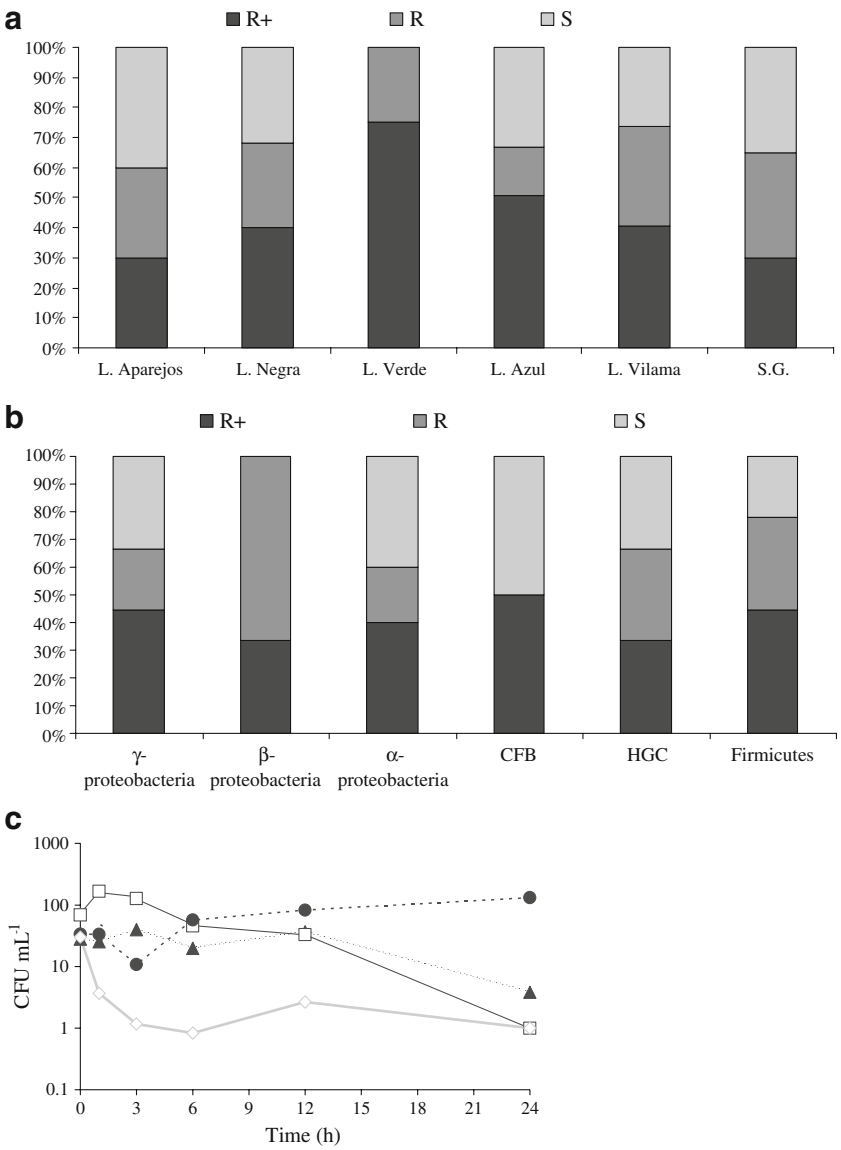


Figure 4 Distribution of different degrees of UV resistance (sensitive, medium, and high) among studied lakes (**a**), among the phylogenetic groups (**b**), and an example of different classes of UVR resistance of strains isolated from L. Vilama (**c**). Sensitive (*S*): *Serratia marcescens* V10 (*open diamonds*). Resistant medium (*R*): *Curvibacter lanceolatus* V14 (*open squares*). Highly resistant (*R*+): *S. maltophilia* V11 (*filled triangles*). Highly resistant (*R*+*): *Betaproteobacterium AquaspiA* V15 (*filled circles*)

Denaturing gradient gel electrophoresis (DGGE) was performed using a Bio-Rad Protean II system. Equal amounts of PCR DNA samples were applied directly onto 8% (*w*/*v*) polyacrylamide gels in TAE buffer (40 mM Tris base, 20 mM sodium acetate, and 1 mM ethylenediaminetetraacetic acid) and a linear denaturing gradient of urea and formamide; the concentration of the two denaturants increased from 40% at the top of the gel to 60% at the bottom. Electrophoresis was performed at a constant voltage of 120 V and a temperature of 60°C during 5 h. After electrophoresis, the gel was stained with SYBR® gold nucleic acid gel stain for 10 min, rinsed with TAE buffer, and visualized with UVR in a Bio-Rad Gel Doc 2000.

Distinguishable bands were excised from the gel; eluted DNA was used as template for reamplification using the R518 and F357 primers and these products were sequenced by Macrogen, Korea.

Results

Bacterioplankton Diversity of High-Altitude Lakes

A total of 182 good-quality sequences were obtained from the excised DGGE bands from the isolated bacteria. Most of the DGGE bands shared similarities with uncultured sequences from GenBank and belonged to gammaproteobacteria (42%), *Cytophaga*/*Flavobacterium*/*Bacteroides* (CFB; 18%), Firmicutes (11%), alphaproteobacteria (11%), betaproteobacteria (6%), HGC (2%), and deltaproteobacteria (1%). Total distribution of the

bands sequences included in the main phylogenetic groups from the lakes is presented in Fig. 2a. Figure 2b shows the distribution of the main groups for each lake and Fig. 2c shows the phylogenetic tree of sequences from DGGE bands. Gammaproteobacteria and Bacteroidetes were the most abundant groups in all the studied environments.

In total, 88 isolates were obtained. Most of them presented similarities with uncultured sequences from GenBank and belonged to Firmicutes (42%), gammaproteobacteria (39%), HGC (8%), alphaproteobacteria (6%), betaproteobacteria (3%), and CFB (2%; Fig. 3a). Figure 3b shows the distribution of the main phylogenetic groups among the studied environments and Fig. 3c shows the phylogenetic tree of sequences from isolated strains.

Resistance to UV-B Radiation

A representation that shows how UV-B resistances were calculated in bacteria isolated from L. Vilama is presented in Fig. 4c. As can be observed in this figure, *Betaproteobacterium AquaspiA* V15 was the most resistant bacteria and was classified as R+*.

Exposure to UV-B radiation revealed that most isolates were highly resistant (R+): 33.3% of betaproteobacteria, 50% of CFB, 44.5% of gammaproteobacteria, 44.5% of Firmicutes, 40% of alphaproteobacteria, and only 33.3% of the HGC. A high percentage of Firmicutes, betaproteobacteria, and HGC strains (33.3%, 66.6%, and 33.3%, respectively) was medium resistant (R). CFB, HGC, and alphaproteobacteria showed the highest percentage of sensitive strains (S): 50%, 33.3%, and 40%, respectively (Table 2). Gammaproteobacteria and Firmicutes contributed 33.3% and 22.2% of the sensitive strains, respectively. No sensitive strain was found among betaproteobacteria (Table 3 and Fig. 4b).

No relationship was observed between resistance to UV-B radiation and salinity of the lakes (Table 2). R+ strains were isolated from the lakes regardless of their salinity, ranging from 0.4 to 117 ppm (Table 2 and Fig. 4a).

Several R+ strains were able to survive for 24 h and they are marked with an asterisk in Table 3. Among the gammaproteobacteria, there were four strains belonging to *Pseudomonas* sp. (V1, N23, LDe, and LD2), four to *Acinetobacter* sp. (A2, N40, Ver5, and Ver7), one to *Stenotrophomonas maltophilia* (Ver 4), one to *Halomonas* sp. (LCA6), and one to *Marinobacter* sp. (LCA7) that survived 24 h (Table 3). Firmicutes also presented highly resistant strains. Three isolates belonged to *Bacillus cereus* (Ap7 and V12) and *Bacillus thuringensis* (N17), one strain to *Halobacillus profundi* (V17), two strains to *Exiguobacterium* sp. (N24 and N38), one to *Desemzia* sp (Ver1), one to *Staphylococcus* sp. (Ver9), and *Halobacillus* sp. (LD1).

Table 2 Relative distribution of strains with high (R+), medium (R), or no (S) UV resistance isolated from six different lakes, their taxonomic affiliation, and their pigmentation

Degree of resistance	Percentage of isolates	Percentage of isolates from indicated origin						Percentage of isolates with indicated taxonomic affiliation							Percentage of isolates with pigmentation	
								Gram-negative organisms				Gram-positive organisms				
		L. Aparejos $n=10$	L. Negra $n=25$	L. Verde $n=12$	L. Azul $n=6$	L. Vilama $n=15$	S.G. $n=20$	Gammaproteobacteria $n=36$	Betaproteobacteria $n=3$	Alphaproteobacteria $n=5$	CFB $n=2$	HGC $n=6$	Firmicutes $n=36$	P+ $n=45$	P− $n=43$
	$n=88$														
R+	43.2	30	40	75	50	40	35	44.5	33.33	40	50	33.3	44.5	42.2	44.2
R	28.4	30	28	25	16.7	33.3	30	22.2	66.66	20	–	33.3	33.3	24.5	32.6
S	28.4	40	32	–	33.3	26.7	35	33.3	–	40	50	33.3	22.2	33.3	23.2

P+ visible pigmentation, *P−* no visible pigmentation

Table 3 Phylogenetic characterisation of isolated strains from 6 high altitude lakes in Argentina

Phylogenetic affiliation	Identity %	Accession number	Source	Colour	Medium	Resistance level	Reference
Firmicutes							
Bacillus vallesmortis V8	100	AM235883	L. Vilama	Orange	LM	S	Dib et al. [11]
Bacillus vallesmortis V3	100	AM235882	L. Vilama	Orange	LM	R	Dib et al. [11]
Bacillus cereus V12	100	AM765995	L. Vilama	White	LM	R+*	Current paper
Bacillus sp. V13	98	AM765996	L. Vilama	Blue	LM	R+	Current paper
Halobacillus profundi V17	99	AM766000	L. Vilama	Orange	LM	R+*	Current paper
Bacillaceae bacterium N4	99	AM711582	L. Negra	White	LM	R	Current paper
Exiguobacterium sp. N6	99	AM711583	L. Negra	Transparent	LM	S	Current paper
Bacillus pumilus N14	99	AM712182	L. Negra	Yellow-beige	LM	R	Current paper
Bacillus thuringiensis N17	100	AM712183	L. Negra	White	LM	R+*	Current paper
Bacillus thuringiensis N19	98	AM712184	L. Negra	Pink	LM	R	Current paper
Exiguobacterium sp. N24	99	AM903335	L. Negra	Orange	LM	R+*	Current paper
Exiguobacterium sp. N26	99	AM778698	L. Negra	Orange	LM	R+	Current paper
Bacillus sp. N29	98	AM778699	L. Negra	White	LM	R	Current paper
Exiguobacterium sp. N30	99	AM903336	L. Negra	Orange	LM	S	Current paper
Bacillus cereus N31	99	AM778700	L. Negra	Red-creamy	LM	R	Current paper
Exiguobacterium sp. N34	99	AM778693	L. Negra	Orange	LM	S	Current paper
Exiguobacterium sp. N35	99	AM903337	L. Negra	Orange	LM	R+	Current paper
Bacillus sp. N36	99	AM778694	L. Negra	Creamy-White	LM	R+	Current paper
Bacillus sp. N37	99	AM778695	L. Negra	Creamy-White	LM	R	Current paper
Exiguobacterium sp. N38	99	AM903338	L. Negra	Orange-Pink	LM	R+*	Current paper
Exiguobacterium sp. N39	99	AM903339	L. Negra	Strong Orange	LM	S	Current paper
Bacillus pumillus A4	99	DQ217665	L. Azul	White	LM	S	Dib et al. [11]
Staphylococcus saprophyticus A3	97	DQ112023	L. Azul	Soft orange	LM	R	Dib et al. [11]
Bacillus cereus Ap4	99	AM711589	L. Aparejos	White	LM	R+	Current paper
Bacillus cereus Ap7	99	AM711591	L. Aparejos	White	LM	R+*	Current paper
Staphylococcus sp. Ap10	100	AM711593	L. Aparejos	Transparent	LM	R	Current paper
Bacillus cereus Ap11	98	AM711594	L. Aparejos	White	LM	R	Current paper
Desemzia sp Ver1	99	AM778684	L. Verde	Yellow-White	LM	R+*	Current paper
Staphylococcus sp. Ver2	100	AM778685	L. Verde	Orange	LM	R+	Current paper
Staphylococcus sp. Ver9	100	AM778692	L. Verde	Orange-creamy	LM	R+*	Current paper
Staphylococcus sp. Ver11	100	AM903333	L. Verde	Orange-creamy	LM	R+	Current paper
Bacillus sp. LAM1	99	FM865886	S.G.	White	LM	R	Current paper
Staphylococcus sp. *LCA*	98	FM865889	S.G.	Blue-White	10% MGM	S	Current paper
Halobacillus sp. *LD1*	99	FM865898	S.G.	White	10% MGM	R+*	Current paper
γ-proteobacteria							
Pseudomonas sp. V1	98	AM403128	L. Vilama	White	LM	R+*	Dib et al. [11]
Enterobacter V4	98	AM403125	L. Vilama	White	LM	S	Dib et al. [11]
Serratia marcescens V10	95	AM765993	L. Vilama	Pink	LM	S	Current paper
S. maltophilia V11	98	AM765994	L. Vilama	Yellow	LM	R+	Current paper
Salinivibrio costicola V16	94	AM765999	L. Vilama	Pink	LM	R	Current paper
Acinetobacter sp. N40	99	AM778696	L. Negra	Bluish creamy	LM	R+*	Current paper
S. maltophilia N7	99	AM711584	L. Negra	Orange	LM	S	Current paper
Pseudomonas sp. N23	100	AM778697	L. Negra	Yellow-transparent	LM	R+*	Current paper
Pseudoalteromonas sp. N32	98	AM778701	L. Negra	White-Grey	LM	R+	Current paper
Burkholderia cepacia N12	99	AM711586	L. Negra	White-Yellow	LM	S	Current paper
Acinetobacter johnsonii A2	99	AY963294	L. Azul	White	LM	R+*	Dib et al. [11]
Pseudomonas plecoglossicida Ap1	99	AM711587	L. Aparejos	Blue-White	LM	S	Current paper
Pseudomonas plecoglossicida Ap3	99	AM711588	L. Aparejos	Orange	LM	S	Current paper
Pseudomonas plecoglossicida Ap17	99	AM711596	L. Aparejos	White	LM	S	Current paper
Acinetobacter sp. Ver5	99	AM778688	L. Verde	Creamy-White	LM	R+*	Current paper

Table 3 (continued)

Phylogenetic affiliation	Identity %	Accession number	Source	Colour	Medium	Resistance level	Reference
Acinetobacter sp. Ver3	98	AM778686	L. Verde	Transparent-Blue	LM	R	Current paper
Acinetobacter junii Ver7	98	AM778690	L. Verde	Orange	LM	R+*	Current paper
Stenotrophomonas sp. Ver4	97	AM778687	L. Verde	Transparent-yellow	LM	R+*	Current paper
Pseudomonas sp Ver6	98	AM778689	L. Verde	Transparent	LM	R	Current paper
Stenotrophomonas sp. Ver8	96	AM778691	L. Verde	Transparent	LM	R	Current paper
S. maltophilia Ver10	98	AM903332	L. Verde	Creamy-Yellow	LM	R+	Current paper
S. maltophilia Ver12	99	AM903334	L. Verde	Transparent-Blue	LM	R+	Current paper
Stenotrophomonas sp. LAP1	87	FM865888	S.G.	Brown	LM	R+	Current paper
Pseudomonas sp. LAM2	99	FM865887	S.G.	Transparent-Blue	LM	R	Current paper
Aeromonas sp RP3	99	FM865884	S.G.	Blue-White	LM	R	Current paper
Stenotrophomonas sp. RP5	77	FM865885	S.G.	Yellow	LM	S	Current paper
Marinobacter sp. LCA6	99	FM865892	S.G.	Blue-White	10% MGM	R+*	Current paper
Halomonas sp. LCA7	99	FM865893	S.G.	Creamy-White	10% MGM	R+*	Current paper
Stenotrophomonas sp. LDc	99	FM865895	S.G.	Transparent-White	LM	R	Current paper
Pseudomonas sp. LDd	98	FM865896	S.G.	Yellow	LM	S	Current paper
Pseudomonas sp. LDe	98	FM865897	S.G.	Creamy-White	LM	R+*	Current paper
Halomonas sp.LD2	97	FM865899	S.G.	Blue	10% MGM	R+*	Current paper
Acinetobacter sp. LCE1	99	FM865881	S.G.	White	LM	S	Current paper
Acinetobacter sp. LCE2	99	FM865882	S.G.	While	LM	R	Current paper
Halomonas arcis LD3	98	FM865883	S.G.	Creamy-Yellow	10% MGM	S	Current paper
β-proteobacteria							
Curvibacter lanceolatus V14	97	AM765997	L. Vilama	White	LM	R	Current paper
Beta proteobacterium AquaspiA V15	97	AM765998	L. Vilama	Iridescent	LM	R+*	Current paper
Burkholderia cepacia Ap8	99	AM711592	L. Aparejos	Yellow	LM	R	Current paper
α-proteobacteria							
Sphingomonas sp. N3	99	AM711581	L. Negra	Yellow	LM	R	Current paper
Sphingomonas sp. N9	99	AM711585	L. Negra	Yellow-dark	LM	S	Current paper
Caulobacter sp. N13	82	AM712181	L. Negra	Yellow	LM	R+*	Current paper
Sphingomonas sp. Ap5	99	AM711590	L. Aparejos	Yellow-Dark	LM	R+*	Current paper
Agrobacterium tumefaciens LC1	99	In process	S.G.	Transparent-White	LM	S	Current paper
HGC							
Micrococcus sp. A1	98	AM403127	L. Azul	Yellow	LM	S	Dib et al. [11]
Nocardia sp. A5	99	DQ112024	L. Azul	Strong orange	LM	R+*	Dib et al. [11]
Micrococcus sp. A7	99	AM235879	L. Azul	Yellow	LM	R+*	Dib et al. [11]
Micrococcus sp. V7	98	AM403126	L. Vilama	Yellow	LM	R	Dib et al. [11]
Rhodococcus eritropolis V2	97	AM236137	L. Vilama	White	LM	S	Dib et al. [11]
Brachybacterium sp. V5	97	AM236138	L. Vilama	Yellow	LM	R	Dib et al. [11]
Kocuria sp. N33	100	AM778702	L. Negra	Red- Strong	LM	S	Current paper
Brevibacterium sp. Ap13	99	AM711595	L. Aparejos	Blue-White	LM	S	Current paper
Micrococcus sp. LCA5		FM865891	S.G.	Yellow	10% MGM	R	Current paper
Bacteroidetes							
Psychroflexus sp. LCA4	94	FM865890	S.G.	Orange	10% MGM	R+*	Current paper
Psychroflexus sp. LCA9	97	FM865894	S.G.	Pink	10% MGM	S	Current paper

* Strains marked with an * (R+*) were highly resistant and able to grow after 12h of exposure

Highly resistant alphaproteobacteria belonged to *Caulobacter* sp. (N13) and *Sphingomonas* sp. (Ap5). The HGC group presented two highly resistant bacteria: *Nocardia* sp. (A5) and *Micrococcus* sp. (A7). In addition, betaproteobacteria and Bacteroidetes groups that presented one strain, *Psychroflexus* sp. (LCA4) and *Betaproteobacterium AquaspiA* (V15), respectively, were also classified as highly resistant (R+*).

Pigmentation of Isolates

The number of pigmented and nonpigmented strains was rather similar (45 and 43, respectively). The majority of pigmented strains (42.2%) was highly resistant (R+). However, pigmented and nonpigmented R+ strains were equally distributed (42.2% and 44.2%, respectively). Pigmented strains were less represented in medium resistant isolates (R) than nonpigmented strains (24.4% against 32.6%, respectively; Table 2). Among sensitive strains, the number of pigmented strains was higher than nonpigmented strains (33.3% against 23.2%, respectively).

Discussion

Gammaproteobacteria were predominant in the total community of the six Andean lakes (42% of DGGE bands) and isolates (39% of the isolates). They also presented the highest UV resistance compared with other phylogenetic groups (Fig. 4b). Similar results have been found in marine bacterioplankton [1, 3]. *A. johnsonii* and *Pseudomonas* sp. have been previously proposed by our research group as highly UV-resistant bacteria [12, 48] and these results have been confirmed in strains isolated in the current study. *S. maltophilia* is a widely distributed group in extreme environments with high antibiotic resistance (data not shown) and this bacterium also demonstrated to be highly UV-resistant.

CFBs were abundant in the total community (18% of DGGE bands) and abundance of this group was also found by Demergasso et al. in Chilean Andean lakes [9]. However, only few isolates were obtained in the present study and thus UV-B resistance for the phylogenetic group could not be established.

Firmicutes were abundant in the total community (11% of DGGE bands) and predominant among isolates (42%). They presented the highest UV resistance. *Exiguobacterium* sp. was widely distributed in the saline environments and presented high UV-B resistance.

Betaproteobacteria were little represented among isolates (3%) and DGGE bands (6%), and this can be explained by the saline conditions of most of the lakes studied. Due to the low number of isolates, the high UV-B resistance detected cannot be considered representative of the whole group.

Alphaproteobacteria represented 11% of total DGGE bands, probably also due to salinity. Isolates (6% of the total) presented high UV-B resistance. UV resistance in this phylogenetic group was previously reported by our research group [14].

Resistance of Halophilic Versus Nonhalophilic Strains

The underlying hypothesis of the present study is that bacteria isolated from more saline environments would be more resistant to solar radiation than those isolated from less saline environments because saline stress would induce oxidative stress similar UV stress. Thus, adaptive strategies developed to resist saline stress would be similar to those necessary to face UV stress. However, no relationship was found between UV-B resistance of the isolates and the biotope from which they were isolated, i.e., a hypersaline (L. Vilama or SG) or oligosaline (L. Aparejos or L. Verde) lake (Fig. 4a). These observations suggest that resistance to radiation is well-distributed among bacterial species present in high-altitude wetlands. Similarly, no significant differences in decrease of bacterioplankton diversity were found in studies performed in water from lakes with different levels of salinity after irradiation with UV-B.

Role of Pigmentation in Resistance to UV-B Radiation

Carotenoids have been proposed to protect microorganisms from UV and visible light damage by quenching triplet-state photosensitizers and reactive oxygen species [6, 30]. Many reports suggest that pigments in bacteria are effective against solar radiation [22, 26, 47]. However, the present study gives clear evidence that there is no direct correlation between pigmentation, high UV-B resistance, and the occurrence of pigmented bacteria in the high Andean lakes. Therefore, pigmentation may have only an indirect effect on the resistance of bacterial cells against solar radiation, probably throughout antioxidant activity. Similar results were previously found by our research group in Andean isolates [16, 17] and by other authors in marine isolates [1].

Conclusion

Our results demonstrate that (1) gammaproteobacteria were predominant in bacterial diversity, (2) they presented high UV-B resistance, and (3) distribution of resistant bacterial isolates was independent of the salinity of the isolation source (lake). Furthermore, they have showed (4) a large interspecific variability of resistance to UV-B radiation and (5) the lack of a direct relationship between pigmentation and UV resistance.

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