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Full Paper

Isolation of UV-B resistant bacteria from two high altitude Andean lakes (4,400 m) with saline and non saline conditions

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Laguna (L.) Negra and L. Verde are high altitude Andean lakes located at the 4,400 m altitude in the Andean desert (Puna) in the Argentine northwest. Both lakes are exposed to extreme weather conditions but differ in salinity contents (salinity 6.7% for L. Negra and 0.27% for L. Verde). The aim of this work was to isolate ultraviolet B fraction (UV-B) resistant bacteria under UV-stress in order to determine, a possible connection, between resistance to UV-B and tolerance to salinity. DNA damage was determined by measuring CPDs accumulation. Connection among pigmentation production and UV resistance was also studied. Water samples were exposed to artificial UV-B radiation for 24 h. Water aliquots were plated along the exposition on different media, with different salinity and carbon source content (Lake medium (LM) done with the lake water plus agar and LB). CFU were counted and DNA damage accumulation was determined. Isolated bacteria were identified by 16S rDNA sequence. Their salinity tolerance, were measured at 1, 5 and 10% NaCl and their pigment production in both media was determined. In general it was found that UV resistance and pigment production were the optimum in Lake Medium done with lake water which maintained similar salinity. The most resistant bacteria in L. Negra were different strains of *Exiguobacterium* sp. and, in L. Verde, *Staphylococcus* sp. and *Stenotrophomonas maltophilia*. These bacteria showed the production and increase of UV-Vis absorbing compounds under UV stress and in LM. Bacterial communities from both lakes were well adapted to high UV-B exposure under the experimental conditions, and in many cases UV-B even stimulated growth. The idea that resistance to UV-B could be related to adaptation to high salinity is still an open question that has to be answered with future experiments.

Key Words——Andean lake microbiology; extreme environments; microbial community; UV-B resistance

Introduction

Laguna (L.) Verde salt flat is a system of five shallow, oligotrophic lakes originated in the Tertiary geological period (65 million to 1.8 million years ago) and it is distributed over 100 km^2 of High Andean Altiplane (Fig. 1). These lakes are located between 4,200 and 4,400 m above sea level and distances between them vary from 10 to 20 km. Characteristics of L. Negra and L. Verde are given in Table 1. Almost unexplored, with no access roads, these aquatic ecosystems endure

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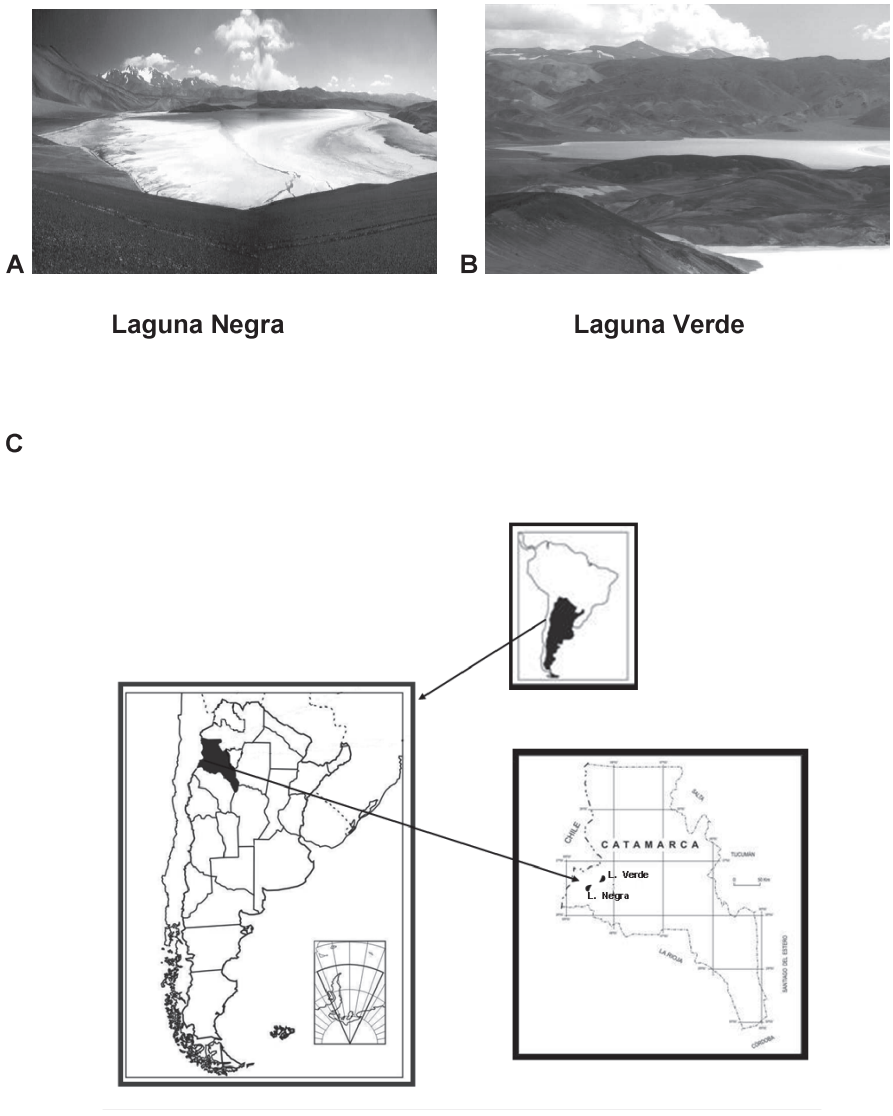

Fig. 1. Ecosystems of High Altitude Andean Lakes in north western Argentinean Andes.

(A) L. Negra (4,400 m), (B) L. Verde (4,400 m), (C) Geographic location of the Laguna Verde saltflat.

extreme environmental conditions such as high levels of ultraviolet B fraction (UV-B) radiation (Fernández Zenoff et al., 2006a), high salinity levels, low nutrients (particularly phosphorous), heavy metal contents (mainly arsenic) (Mantelli et al., 2003) and large daily temperature fluctuations. Due to the high altitude and the geographical and physicochemical characteristics of these lakes, UV-B radiation is one of the most limiting abiotic factors for bacterioplankton communities (Agogué et al., 2005; Alonso-Saez et al., 2006; Fernández Zenoff et al., 2006b; Hernández et al., 2007; Wilson et al., 2004; Winter et al., 2001).

Bacteria are especially sensitive to UV radiation because of their simple haploid genomes with low or nonfunctional redundancy, the lack of a protective cell wall and their diminutive size (Garcia-Pichel, 1994). According to biological responses, UV radiation can be divided into three bands according to the wavelength: UV-C $<$ 280 nm; UV-B = 280−320 nm and UV-A = 320−400 nm. High doses of UV radiation, especially those from band B, would damage the cell directly and indirectly, because of the strong absorption by DNA molecules at wavelengths below 320 nm (Boelen et al., 2000; Mitchell and Karentz, 1993). Accumulation of

Table 1. Characteristics of L. Negra and L. Verde from Salar Laguna Verde, Catamarca, Argentina.

Lake	L. Negra	L. Verde
Global Position	27° 34′ S; 65° 32′ W	27° 38′ S; 68° 32′ W
Altitude (masl)	4,400	4,400
Arsenic (mg/L)	3	0.8
Phosphor (mg/L)	$<0.05^{*}$	$<0.012^{*}$
Salinity (%)	32	5
Chlorophyll (μg/L)	0.63	1.04
Maximum UV-B (W/m^2) in situ 280–312 nm	10.80	10.78

cyclobutane pyrimidine dimers (CPDs) in DNA is most commonly observed after irradiation with UV-B (Fernández Zenoff et al., 2006b).

In addition to the stress imposed by UV radiation, organisms living in some of these environments must also deal with high salinity, desiccation-rehydration cycles and high levels of other mutagenic agents. In that way, there are hypotheses that propose that a broad phylogenetic spectrum of microorganisms which are adapted to stress by desiccation, high salinity or exposute to metals, can also be adapted to radiation stress, since both stresses have deleterious effects for the cells, in particular on DNA, causing a strong oxidative stress (Agogué et al., 2005; Alonso-Saez et al., 2006). Thus, mechanisms of tolerance to different stresses could then be expected in all of these environments. In that way, halophilic and extremophilic eubacteria are thought to have developed mechanisms that produce a high degree of resistance to high doses of these DNA-damaging agents (Martin et al., 2000; Wilson et al., 2004).

In other hand it is known that UV radiation is responsible of biological damage produced by the formation of Reactive Oxigen Species (ROS) and DNA damage. ROS can affect proteins and membranes by lipoperoxidation. As a cellular protective response, several bacterial strains produce metabolites such as photoprotective compounds that could protect to DNA from UV radiation damage or carotenoids that reacted with ROS to neutralize their harmful effect.

In order to determine the relationship between UV-B resistance, colored compounds production and salinity tolerance, we aim to test the UV-B resistance of bacteria isolated from lakes with similar UV-B doses but different salinity conditions. For this purpose L. Negra and L. Verde were chosen, because both lakes are located at the same altitude and share similar environmental conditions but they differ in salinity content (Table 1).

In addition, in this report, a preliminary exploration of bacterial diversity of two different unexplored extreme environments is presented. The discovery and isolation of different bacterial species, the study of CPD accumulation and discussion about the possible role of bacteria pigmentation in UV-B resistance are considered.

Materials and Methods

Sampling. Surface water samples of both lakes were collected during the late winter and early spring of 2006 (September) in 10 L acid-washed polyethylene containers, after pre-rinsing with lake water. Water samples were stored at 4°C until further processing at the laboratory (within 24 h after collection).

In vitro radiation experiments. To determine the effect of UV-B on bacterioplankton from L. Negra and Verde, water samples (300 ml) were transferred to UVR transparent containers (Plexiglas) of 800 ml capacity. Then, the water samples were exposed to UV-B radiation ($I=1.92$ W/m^2) during 24 h with artificial lamps (two 09815-06 lamps, Cole Parmer Instruments Company, with the major emission line at 312 nm) and temperature was controlled (15°C). Radiation assays were carried out in duplicate and dark controls were included for all experiments. UV-B lamps were covered with acetate film to block out UV-C and the amount of UV-B radiation was measured using a radiometer (09811-56, Cole Parmer Instruments Company) at 312 nm with an intensity of 1.9 W/m^2. Aliquots (20, 25 ml) were extracted at different exposure times (1, 3, 6, 12 and 24 h) to determine: culturable bacteria (identified by the rRNA 16S sequence) and DNA damage (by measuring CPD accumulation).

In order to discard a possible effect of an enclosed sample ('bottle effect'), results obtained were compared with dark controls: that means that the same experiments (the same exposure time and the same assay temperature) were carried out with bottles wrapped in aluminum paper.

Comparing UV-B exposed samples with unexposed (dark control), 3 classes of performance were determined: i) bacteria that diminished their cell number under UV-B, compared to the control, were classified as sensitive (S), ii) bacteria that maintained the same cell count as the control were classified as resistant (R), iii) bacteria that increased their cell number compared to the control or bacteria that were isolated only under UV-B irradiation were classified as super resistant (R^+).

Bacterial count isolation and growth. Twenty ml were concentrated by centrifugation (15 min at 13,000 rpm) and resuspended in 1 ml sterile water. One hundred μl of 20× concentrated water samples were plated in duplicate onto two culture media with different carbon sources: Luria Broth (LB) and Lake Medium (LM). LB was used to select frequent culturable bacteria and LM to maintain the same salinity as the isolation environment. The latter was made with filtered lake water (0.22 μm Biopore filters) supplemented with 0.25% (w/v) yeast extract and 1.4% (w/v) agar (Difco). Plates were incubated at 20°C for 24 h and 72 h for LB and LM, respectively.

To determine the behavior of different bacteria during UV-B exposure, all isolates were classified on agar plates by color, colony surface and size, phenotypically different bacteria were grouped, counted, isolated and identified by 16S rRNA analysis. Internal transcribed spacer (ITS) analysis was carried out in order to discriminate between similar strains (data not shown). All the isolates color, affiliation and UV resistance are represented in Table 2, and Fig. 2. This procedure allowed determination of the number and diversity of culturable bacteria present in the lakes after artificial UV-B radiation.

To analyze changes in metabolites production relat-

Table 2. Phylogenetic characterization of L. Negra and L. Verde water bacteria isolated during in vitro UV-B.

Strain	Medium	Color	Closest relative species in the 16S rDNA gene sequence database	Accession number	% Sequence similarity	UV- B Radiation sensitivity	Dark control
			L. Negra				
N22	LM	White	*Exiguobacterium* sp. 1.	In process	99	S	+
N23	LM	Yellow	*Pseudomonas* sp.	AM778697	100	R^+	+
N24	LM	Orange	*Exiguobacterium* sp. 2	AM903335	100	R	+
N26	LM	Orange	*Exiguobacterium* sp. 3	AM778698	99	S	+
N29	LM	Uncolored	*Bacillus* sp.	AM778699	98	R^+	+
N31	LM	Pink	*Bacillus cereus*	AM778700	99	R^+	−
N32	LM	Gray	*Pseudoalteromonas* sp.	AM778701	98	R^+	−
N33	LM	Red	*Kocuria* sp.	AM778702	100	S^-	+
N36	LB	Uncolored	*Bacillus* sp.	AM778694	99	S	+
N38	LB	Orange-pink	*Exiguobacterium* sp.	AM903338	99	R	+
N40	LB	Blue	*Acinetobacter lwoffii*	AM778696	99	R	+
			L. Verde				
Ver5	LM	Uncolored	*Acinetobacter* sp.	AM778688	99	R	+
Ver6	LM	Phosphorescent	*Pseudomonas* sp.	AM778689	9	S	+
Ver7	LM	Orange	*Acinetobacter junii*	AM778690	98	S	+
Ver12	LM	Uncolored	*Stenotrophomonas maltophilia*	AM903334	96	R	+
Ver9	LM	Orange	*Staphylococcus* sp.	AM778692	100	R^+	−
Ver1	LB	Yellow-white	*Desemzia incerta*	AM778684	99	R	+
Ver2	LB	Blue	*Staphylococcus* sp.	AM778685	100	S	+
Ver3	LB	Blue	*Acinetobacter johnsonii*	AM778686	98	R	+
Ver4	LB	Uncolored	*Stenotrophomonas maltophilia*	AM778687	97	R	+

References: Isolated compared with presence (+) or absence (−) in dark control. R^+: super resistant; R: resistant; S: sensitive, S^-: super sensitive (bacteria that were isolated only in dark incubation).

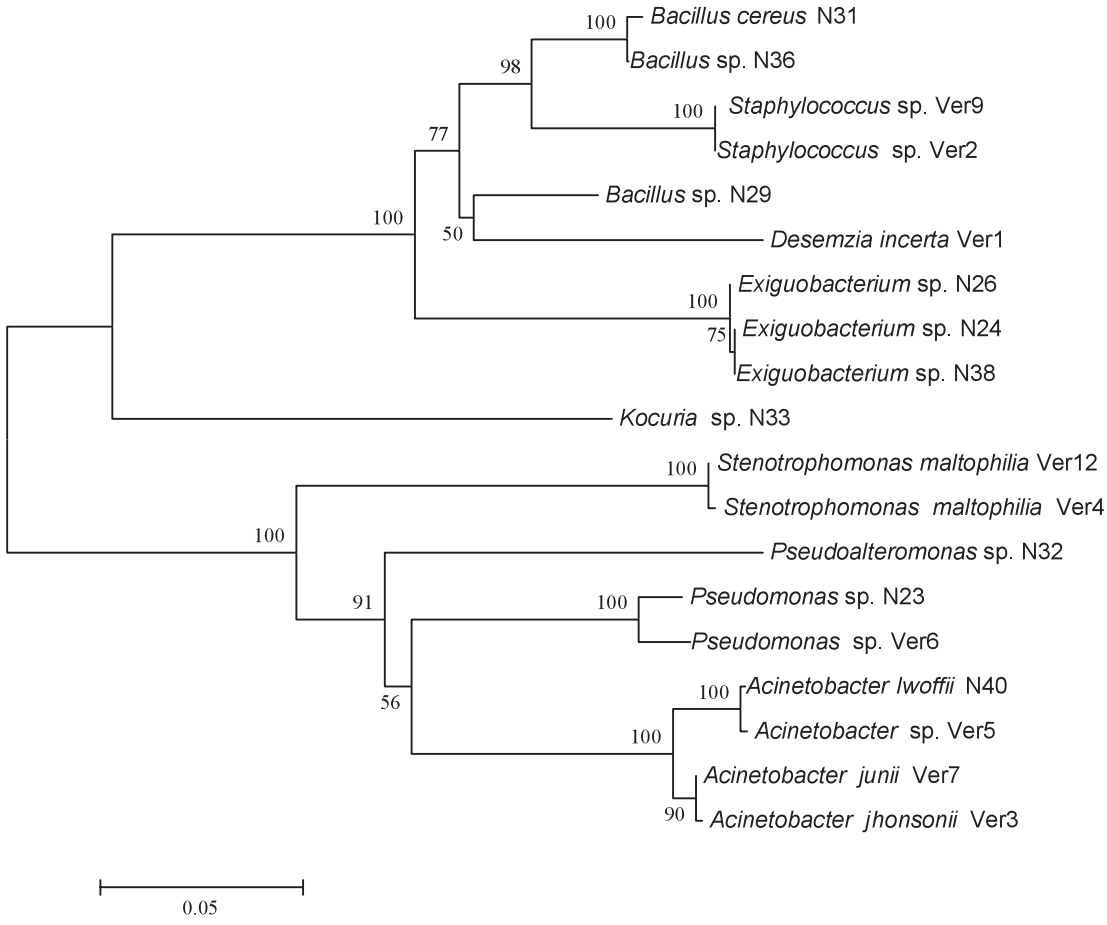

Fig. 2. Neighbor-joining tree representing the phylogenetic relationship among partial sequences of isolated strains from L. Verde and L. Negra. Bootstrap values are shown at the branching points (percentage of 1,000 replicates).

ed to growth medium and UV resistance, isolated strains were grown to stationary phase (when pigmentation is visible) in two culture media with different salinity content (LM and LB). Culture were diluted (with NaCl solution sterile 0.9%) to an Optical Density (OD) of 0.7. Then, the cultures were irradiated for 1 h (Dosis: 6.9 kJ/m^2). Five milliliters of diluted culture were taken and concentrated by centrifugation (5 min to 10,000 rpm). Pellet was extracted with 2 ml of MeOH to measure their absorption spectra (250−750 nm) using an Espectrophotometer Beckman DU640 UV-VIS.

Salinity effect on bacterial growth. Growth curves of selected strains in different salinity conditions were done by incubating at 30°C in flask with shaking in LB with 1, 5, 10 and 15% NaCl. OD_{600} was measured during 24 h with intervals of 5 h. Specific growth rates were calculated from $\mu = (\ln OD_{600}t_2 - \ln OD_{600}t_1)/ t_2 - t_1$; $t_2 > t_1$ where time 1 (t_1) and time 2 (t_2) are the extremes of exponential phase in growth curve.

Nucleic acid extraction and quantification and determination of phylogenetic affiliation of isolated bacteria. Bacterial genomic DNA of each isolated strain was extracted with the CTAB extraction method and 16S rRNA amplification was carried out using standard protocols described by Fernández Zenoff et al. (2006a). Genotypic affiliation of isolated strains was performed with single colonies. Universal primers 27 F and 1492 R were used to amplify the 16S rDNA gene. PCR products were checked on 0.8% (w/v) agarose gels and DNA sequencing was performed by Macrogen Inc. (Korea). The sequences were registered in the GenBank Database (Table 2).

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 (Thompson et al., 1994). Analyses were performed by the neighbor-joining method using the MEGA 4.0 software (Saitou and

Nei, 1987; Tamura et al., 2007). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown above the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) 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).

ITS determination. The ITS region was amplified using primers to positions 1492−1510 of the 16S rRNA gene (*SM, 5′-AAGTCGTAACAAGGTAGCC-3′) and positions 482-463 of the 23S rRNA gene (BR3, 5′-GC TTTTCACCTTTCCCTCAC-3′). Amplification was carried out as described by Willems et al. (2001). For each sample, amplification was done as follows: twenty-five μl reaction volume contained 0.125 μl *Taq* polymerase (Promega), reaction buffer 5 × (100 mM Tris/HCl pH 8.8, 500 mM KCl, 0.8% [v/v] NP-40 and 1.5 mM $MgCl_2$), 2 pmol of each of the primers (final vol.), 0.2 mM of each of the four dNTPs (MBI Fermentas) and 1 μl of bacterial DNA.

The PCR protocol consisted of an initial denaturation step of 5 min at 94°C, followed by 35 denaturation cycles of 2 min at 94°C, 1 min annealing at 59°C and 1.5 min at 72°C, and a final extension of 7 min at 72°C. Amplification products were analyzed by 0.8% (w/v) agarose gel electrophoresis.

Quantification of CPDs. Twenty-five ml of water samples were filtrated by 0.22 μm pore size and DNA extracted by the CTAB method. The amount of CPDs was determined using the H3 antibody with a high affinity for thymine dimers according to George et al. (2002). Briefly, heat-denatured DNA samples (100 ng) were blotted (Minifold I SRC96D dotblot device, Schleicher & Schuell) onto nitrocellulose membranes (Schleicher & Schuell, Protran 0.1 μm). The membranes were baked at 80°C for 2 h to immobilize DNA and incubated in 5% (w/v) skimmed-milk powder in PBS-T (PBS + 0.1% [v/v] Tween 20 [Sigma]), for blocking unspecific sites, for 30 min at room temperature. After three washing steps with PBS-T, the membranes were incubated overnight with the primary antibody H3 at 4°C. After repeated washing, incubation with the secondary antibody (Polyclonal Rabbit Anti-Mouse Immunoglobulins/HRP, DakoCytomation) was carried out for 2 h at room temperature. Detection of CPDs was obtained using ECL detection reagents (Amersham) in combination with photosensitive films (Amersham Hyperfilm ECL). Finally, the films were scanned and analyzed using an image analyzer (Gel Doc 2000 Bio Rad). To quantify the amount of damage in the sample DNA, samples were compared with a calibration series of standard DNA (Promega). Calf thymus DNA was used as a biodosimeter, to compare the DNA damage among a nacked DNA and DNA in the cell, this allows to determine the efficience of the cell repair or protect mechanisms against UV damage.

Results and Discussion

Survival of culturable bacteria on two different media after UV-B radiation

Assessment of survival of culturable bacteria after 24 h of UV-B exposure was carried out with two plating media in order to enhance isolation conditions: a rich medium (LB) and a minimal medium (LM, prepared with water from the lakes). LM preserved the salinity of the isolation environment (salinity 6.7% for L. Negra and 0.27% for L. Verde). The total number of Colony Forming Units (CFUs) counted on isolation media of both lakes is presented in Fig. 3. Clear differences in UV-B resistance between the two plating media can be noticed. L. Negra showed an increase in the number of CFUs of bacteria after 3 h of exposed to UV-B, plated onto LM, and whereas UV-B exposed bacteria from this lake plated onto LB exhibited a general decrease in the number of CFUs. Regarding L. Verde, a significant increase ($p < 0.05$) was observed after 12 h of ex-

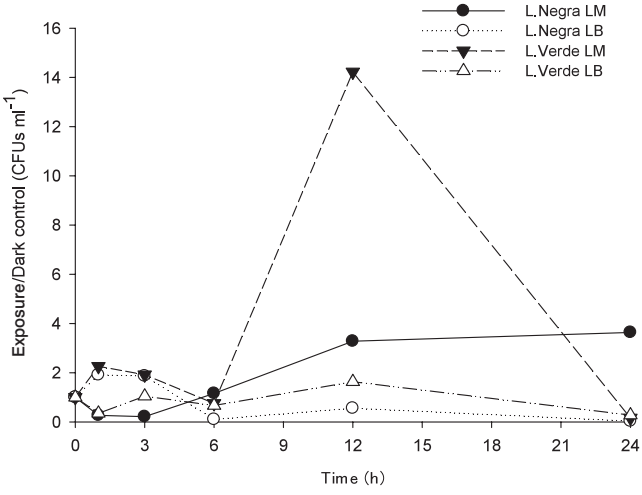

Fig. 3. Survival curves of total isolated strains that compose L. Negra and L. Verde bacterioplankton community during long-term artificial UV-B exposure.

posure to UV-B on LM, but the number of CFUs decreased at the end of the experiment in both media.

The stimulation of bacterial growth with UV-B exposure has already been observed in previous reports on culturable bacteria and in total community analyses from L. Azul and L.Vilama (Dib et al., 2009; Farias et al., 2009; Fernández Zenoff et al., 2006a), situated at 4,450 and 4,650 m altitude, respectively. The increase in the number of cells and diversity under UV-B stress needs deeper studies directed to explain the possibility that UV triggered certain mechanisms that enhanced the ability of cells to survive and replicate faster (McGlynn and Lloyd, 2002).

Identification and differential UV-B resistance of culturable bacteria on LB and LM culture media

More bacterial diversity, better UV resistance and increment in colored compounds were found when samples were plated in LM with respect to LB. It is apparent from this data that LM media which maintains similar isolation source salinity improved bacterial survival under UV radiation.

Different bacteria were isolated with the two media from L. Negra. Isolations from LB included strains of *Exiguobacterium* sp., *Bacillus cereus* and *Acinetobacter lwoffii* (Fig. 4A). Strain *Exiguobacterium* sp. and *Acinetobacter lwoffii* were considered resistant (R) while *Bacillus cereus* were found to be sensitive (S).

Pseudomonas sp., *Bacillus* sp., *Bacillus cereus*, *Kocuria* sp. and *Pseudoalteromonas* sp. and three different strains of *Exiguobacterium* sp. (1, 2 and 3), were isolated from LM (Fig. 4A). *Pseudoalteromonas* sp. and *Bacillus cereus* were isolated exclusively under UV-B exposure, which denotes their high resistance (R^+) together with *Bacillus* sp. and *Pseudomonas* sp. *Exiguobacterium* sp. 2 were resistant (R) and *Kocuria* sp. was exclusively isolated in the dark control so it was considered sensible (S) together with *Exiguobacterium* sp. 1 and 3 (Fig. 4A).

Differential bacteria diversity was obtained from L. Verde samples when platting in different medium. While *Desemzia incerta, Staphylococcus* sp., *Acinetobacter johnsonii* and *Stenotrophomonas maltophilia* were isolated from LB, LM favored the growth of *Acinetobacter* sp., *Pseudomonas* sp., *Acinetobacter junii, Stenotrophomonas maltophilia* and *Staphylococcus* sp. (Fig. 4B). In LB medium *Desemzia incerta, Acinetobacter johnsonii* and *Stenotrophomonas maltophilia* maintained the cell number after 24 h of UV-B irradiation comparing with dark control, so they were considered resistant (R) (Fig. 4B). All strains presented an increase of 1 order of magnitude. In contrast *Staphylococcus* sp. displayed depletion on cell counting with respect to the dark control (S).

Staphylococcus sp., the most sensitive strain isolated in LB, was the most resistant strain on LM. In LM it presented color while in LB it did not. Likewise, this strain was not isolated in dark LM control and as is explained below it produce color only under UV stress. *Acinetobacter junii* and *Pseudomonas* sp. were considered sensitive (S). *Stenotrophomonas maltophilia* and *Acinetobacter* sp. maintained the same bacterial cell counting after 24 h of exposure and were considered resistant (R).

All these results shows that *Exiguobacterium* sp., *Staphylococcus* sp., *Stenotrophomonas maltophilia, Desemzia incerta* and *Acinetobacter johnsonii* were the most resistant bacteria isolated. *Exiguobacterium* sp., isolated from L. Negra, is an alkaliphilic, halotolerant, non-spore-forming Gram-positive bacillus which grew both on LB and LM plates after UV-B exposure. This bacterium had already been isolated as a high UV-B resistant strain from L. Vilama, located at 4,650 m altitude in Jujuy, Argentina (Ordonẽz et al., 2009). This bacterium has also been isolated from extreme environments such as Siberian permafrost, ancient salt deposits of a salt mine and chromate-contaminated soil. *Exiguobacterium* sp. support freezing conditions, UV-BC radiation and high osmotic pressure, among other extreme conditions (Chen et al., 2007; Ponder et al., 2005; Sarangi and Krishnan, 2008). Resistance to UV-B radiation in *Staphylococcus* sp. genera has been reported before (El-Adhami et al., 1994). However, there is no record determining this level of resistance in environmental isolates. This is the first report for UV-B resistance in *Stenotrophomonas maltophilia* or *Desemzia incerta*. In contrast, *Acinetobacter johnsonii* has been mentioned in our previous publications as a high UV-B resistant bacterium, isolated from high altitude lakes (Dib et al., 2008; Fernández Zenoff et al., 2006a, b; Ordoñez et al., 2009). These results have also been confirmed by Denaturing Gradient Gel Electrophoresis analysis of DNA with water samples from other lakes from the same environment (Flores et al., in publication).

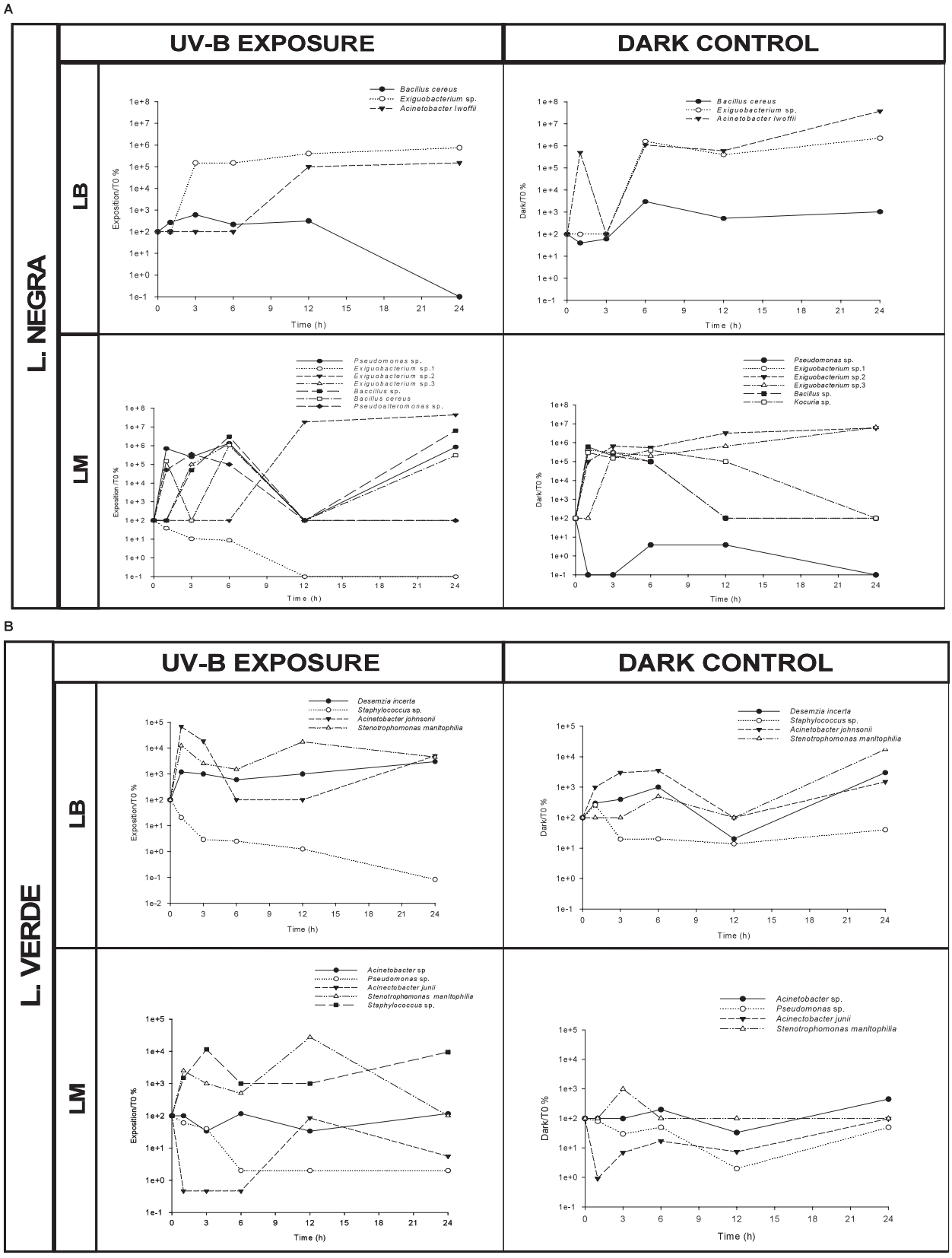

Fig. 4. Isolation of bacteria during exposure compared to dark control in L. Negra (A) and L. Verde (B) bacterioplankton community, during long-term artificial UV exposure plated in two media: LM medium and LB medium.

Salinity tolerance of isolated bacteria

Concerning salinity tolerance it was found that there is no correlation between the capacity of growing in salt and the salinity of the isolation source; nor was found similar salinity tolerance among strains with the same phylogenetic affiliation. *Exiguobacterium* (N22), *Pseudomonas* (N23), *Bacillus* (N29) and *Acinetobacter* (Ver 5) were grouped among high salinity tolerant since they presented low difference in specific growth rates of (less than 10%) among 1% and 5% NaCl (Table 3). Medium salinity tolerance with a ca. 50% depletion of the specific growth rates comparing the growth in 1% and 5% NaCl, was observed in *Exiguobacterium* sp. (N24), *Bacillus cereus* (N31) and *Acinetobacter* sp. (Ver 3). The other tested isolates showed low salinity tolerance. Only *Pseudomonas* sp. (N23) and *Pseudoalteromonas* sp. (N32), were able to growth in 10% salinity, both were isolated from L. Negra that presents a salinity of 6.7%. Connection among salinity tolerance and UV-B resistances could not be established, since UV-B resistant isolates are distributed among salt tolerant and non tolerant group (Table 3).

Production of absorbing compounds in different medium

Bacterial plating after UV-B exposure showed that pigment-producing strains seemed to be more resistant to UV-B than non-producing strains, even with different strains of the same species, as was the case with *Exiguobacterium* sp. and *Bacillus cereus* from L. Negra and *Staphylococcus* sp. from L. Verde. In addition, strains that grew in media that enhanced absorbing compounds production (LM) resulted more resistant than the same strains growing in media that do not enhance absorbing compounds production (LB) (Table 2).

From 20 selected strains, only 3 strains showed differences in their absorption spectra (but without difference in their UV-B resistance), when they were grown in different culture media. *Bacillus cereus* showed an increase in the absorption spectra between 400−500 nm with a max to 470 nm when it was grown in LM comparing LB. This strain was R^+ in LM and was not present in dark control. These absorption maximum is characteristic of carotene pigments such as astaxanthin with known ROS scavenging properties (Asker et al., 2007). Also, the increase in OD below 350 nm (may be ubiquinone, characteristic of *Bacillus* species) was kept when N31 was grown in LM. Similar results were founded in *Exiguobacterium* sp. N38. In a current research (Asker et al., 2007) *Exiguobacterium* sp. was characterized as pigmented-bacteria able to resist radiation; their carotene spectra showed a maximum absorption wavelength to 453 and 480 nm. However, this pigment has not been identified yet at the molecular level.

On the other hand, *Stenotrophomonas maltophilia* showed an higher absorption in their colored compound spectrum when it was grown in LB comparing ML. According to its profile and maximum absorption

Table 3. Specific growth rates of selected strains in LB 1% NaCl, 5% NaCl and 10% NaCl.

Strains	Specific growth rates		
	LB 1% NaCl	LB 5% NaCl	LB 10% NaCl
Gram positive			
Exiguobacterium sp. 2 N24	0.19	0.1	0.06
Exiguobacterium sp. N38	0.42	0.09	0.13
Exiguobacterium sp. 1 N22	0.39	0.35	0.05
Bacillus sp. N36	0.8	0.35	0.07
Bacillus sp. N29	0.22	0.2	0.01
Bacillus cereus N31	0.26	0.16	0.03
Staphylococcus sp. Ver9	0.36	0.19	0.2
Proteobacteria (Gamma)			
Pseudoalteromonas sp. N32	0.49	0.16	0.22
Pseudomonas sp. N23	0.26	0.27	0.25
Acinetobacter lwoffii N40	0.92	0.3	0
Acinetobacter johnsonii Ver3	0.6	0.35	0
Acinetobacter sp. Ver5	0.14	0.14	0.01

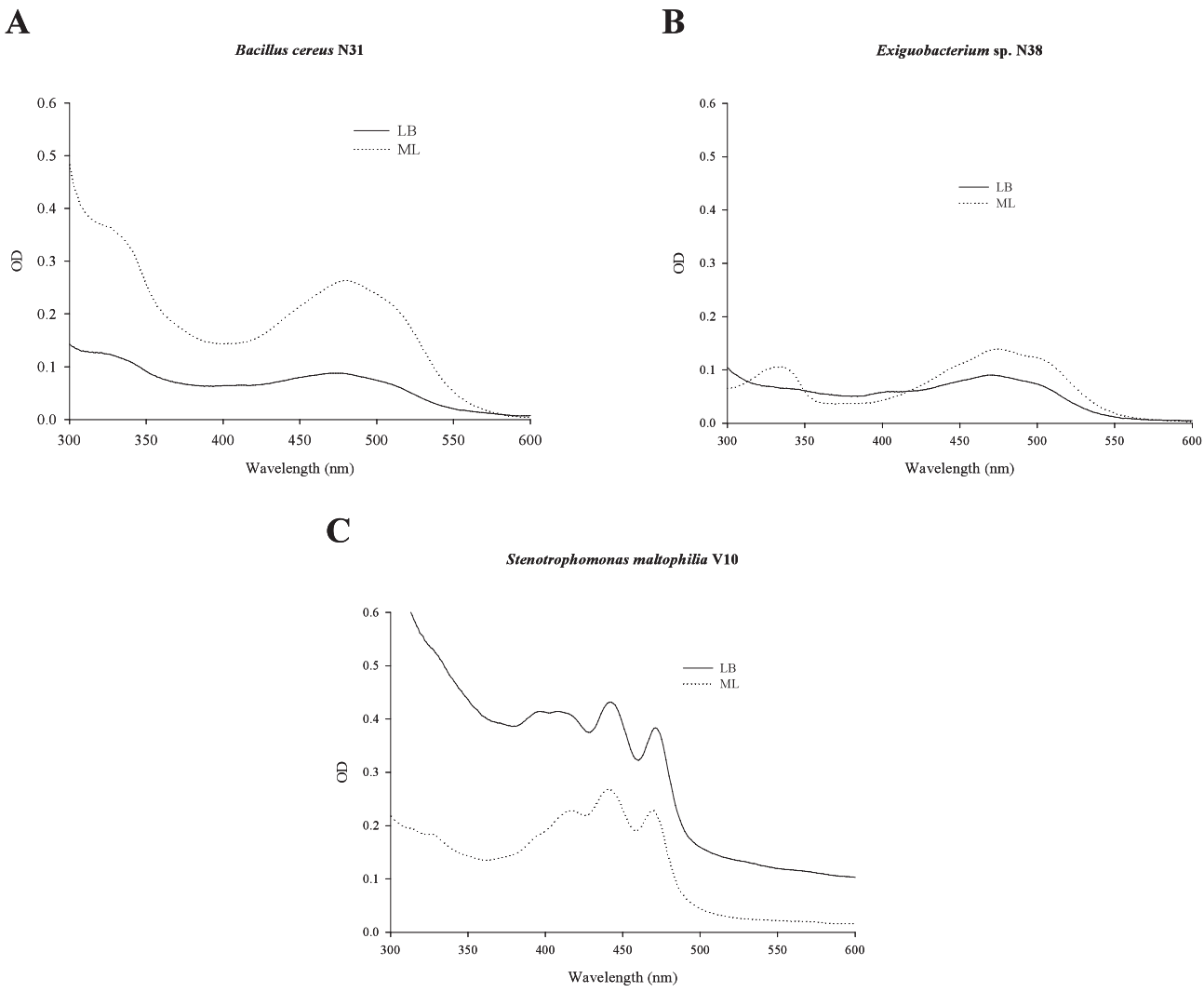

Fig. 5. Absorption spectra of hydrophobic extract, of isolated bacteria growing in both culture media (LB and LM). In this figure it is represented isolates that presented differences among both medium.

wavelength, this strain may have lutein in their pigmentary composition. In fact, it was only isolated in LB (Fig. 5).

Interestingly, *Staphylococcus* sp. Ver 9 isolated from L. Verde water, was the most resistant bacterium to UV-B exposure, and their resistance was only observed after plating onto LM medium and under UV-B exposition. However, we could not be able to detect any absorbing pigmented compound in this strain, nor in LB neither in LM (dark controls). This means that the production of colored compound was induced only in LM under UV-B exposition (ITS analysis revealed that the strains in both media were the same). The reason why this strain exclusively produces pigment under UV stress is an interesting aspect for further research in order to assess the role of pigmentation on UV-B resistance.

In that way we propose that differences found in the colored compounds production, in strains growing in LB and LM demonstrates, that relation among colored compounds production and UV resistance depends on phylogenetic group and conditions of growth (ei carbon source or salinity of the media, UV stress etc.). In that way we proposed that this relationship (colored compound production-UV resistance) can not be generalized. That is why there are studies that associate UV resistance and colored compounds production (Asker et al., 2007) and there are others that do not (Garcia-Pichel, 1994; Ordoñez et al., 2009).

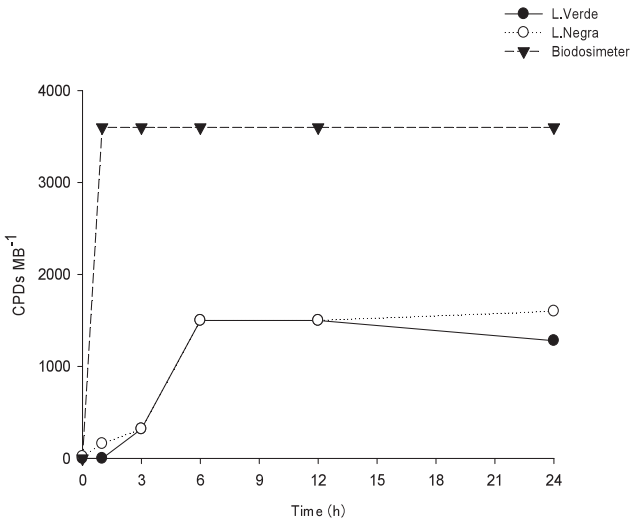

Fig. 6. In vitro CPDs MB^{-1} accumulation in total L. Negra and L. Verde water bacterial community.

Accumulation of CPDs

Total DNA damage in the bacterioplankton community in water from both lakes after UV-B exposure is presented in Fig. 6. Differences in the CPD accumulation between the two samples can be noticed. CPD accumulation in bacteria from L. Negra started after 1 h of exposure (40 CPDs Mb^{-1}) while in bacteria from L. Verde an increase in CPDs was observed after 3 h of exposure. Both samples presented accumulation of 360 CPDs Mb^{-1} in this time. After 3 h of exposure to UVR a logarithmic increase was observed in both samples and maximum CPD accumulation reached around 1,500 CPDs Mb^{-1}. This increase was maintained in L. Negra, while in L. Verde a small decrease was observed until 1,280 CPDs Mb^{-1}. At the same time CPDs in biodosimeters (i.e. calf thymus DNA) were higher than 2,560 CPDs Mb^{-1} from the first moment of sampling (i.e. after 1 h). The maintenance of bacterial counting (mainly in LM), although DNA damage accumulation, demonstrates that, in these environments, DNA damage is well tolerated by the community. In the other hand, the difference in DNA damage presented among bacterial DNA and biodosimeters, demonstrates that bacterial cells efficiently protect their DNA from damage.

Conclusions

From the results discussed, it can be concluded that Andean lake bacterial communities, under the experimental conditions, were well adapted to high UV-B exposure and in many cases UV-B stimulated their growth and selected a different diversity from the original. This phenomenon was more evident in bacteria belonging to L. Negra compared to those from L. Verde and in plating in LM medium that maintained the lake similar salinity. This should be taking in account when the goal is to isolate UV resistant bacteria.

On the other hand, colored compound production demonstrated to be connected to UV exposition, plating medium and phylogenetic group. In that way we want to emphasize that pigmentation in bacteria and UV resistance is not supposed to be generalized and should be studied independently in each phylogenetic group and in different growth conditions. The idea that this resistance could be related to adaptation to extreme environmental factors such as high salinity or high arsenic content is still not clear and will be answered with future experiments.

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References

Agogué, H., Joux, F., Obernosterer, I., and Lebaron, P. (2005) Resistance of marine bacterioneuston to solar radiation. *Appl. Environ. Microbiol.,* **71**, 5282–5289.

Alonso-Saez, L., Gasol, J. M., Lefort, T., Hofer, J., and Sommaruga, R. (2006) Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in northwestern Mediterranean coastal waters. *Appl. Environ. Microbiol.,* **72**, 5806–5813.

Asker, D., Beppu, T., and Ueda, K. (2007) Unique diversity of carotenoid-producing bacteria isolated from Misasa, a radioactive site in Japan. *Appl. Microbiol. Biotechnol*., **77**, 383–392.

Boelen, P., de Boer, M. K., Kraay, G. W., Veldhuis, M. J. W., and Buma, A. G. J. (2000) UV-BR induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic Ocean. *Mar. Ecol. Prog. Ser.*, **193**, 1–9.

Chen, Y. G., Li, H. M., Li, Q. Y., Chen, W., and Cui, X. L. (2007) Phylogenetic diversity of culturable bacteria in the ancient salt deposits of the Yipinglang Salt Mine, P. R. China. *Wei Sheng Wu Xue Bao,* **47**, 571–577.

Dib, J. R., Fernandez-Zenoff, V., Motok, J., Ordoñez, O., and

Farías, M. E. (2008) Occurrence of resistance to antibiotics, UV-B-B and arsenic in bacteria isolated from extreme environment in high altitude Andean wetlands (4,560 m and 4,400 m altitude). *Curr. Microbiol.,* **56**, 510–517.

Dib, J. R., Weiss, A., Neumann, A., Ordoñez, O., Estévez, M. C., and Farías, M. F. (2009) Isolation of bacteria from remote high altitude Andean wetlands able to grow in the presence of antibiotics. *Recent Pat. Anti-Infect. Drug Discov.,* **4**, 66–76.

El-Adhami, W., Daly, S., and Stewart, P. R. (1994) Biochemical studies on the lethal effects of solar and artificial ultraviolet radiation on *Staphylococcus aureus*. *Arch. Microbiol*., **161**, 82–87.

Farias, M. E., Fernández-Zenoff, M. V., Flores, M. R., Ordoñez, O. F., and Estevez, M. C. (2009) Impact of solar radiation on bacterioplankton in Laguna Vilama, a hypersaline Andean saline lake (4,650 m). *J. Geophys. Res.-Biogeosci.*, Special Issue on the High Lakes Project and Other High Altitude Lakes DOI:10.1029/2008JG000784 (in press).

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.

Fernández Zenoff, V., Heredia, J., Ferrero, M. A., Siñeriz, F., and Farías, M. E. (2006a) Isolation of UV-B resistant bacteria from high altitude Andean wetland water. *Curr. Microbiol*., **52**, 359–362.

Fernández Zenoff, V., Siñeriz, F., and Farías, M. E. (2006b) Diverse responses to UV-B radiation and repair mechanisms of bacteria isolated from high altitude aquatic environments (3,600–4,560 m). *Appl. Environ. Microbiol.*, **72**, 7857–7864.

Garcia-Pichel, F. (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreen. *Limnol. Oceanogr.,* **39**, 1704–1717.

George, A. L., Peat, H. J., and Buma, A. G. (2002) Evaluation of DNA dosimeter to assess ozone-mediated variability of biologically harmful radiation in Antarctica. *J. Photochem. Photobiol*., **76**, 274–280.

Hernández, K., Helbling, E. W., Quiñones, R. A., Farias, M. E., and Daneri, G. (2007) UV-B-solar radiation modulates daily production and DNA damage of marine bacterioplankton from productive upwelling zone (36°S). *J. Exp*. *Mar. Biol. Ecol.*, **343**, 82–95.

Mantelli, F., Scala, C., Ronchi, A., Gatti, A., and Minoia, C. (2003) Macrocostituenti ed elementi in traccia nelle acque dei laghi salini delle Andi de Catamarca e La Rioja (Argentina). *Boll. Chim. Igien*., **54**, 37–44.

Martin, E. L., Reinhardt, R. L., Baum, L. L., Becker, M. R., Shaffer, J. J., and Kokjohn, T. A. (2000) The effects of ultraviolet radiation on the moderate halophile *Halomonas elongata* and the extreme halophile Halobacterium salinarum. *Can. J. Microbiol*., **46**, 180–187.

McGlynn, P. and Lloyd, R. G. (2002) Recombinational repair and restart of damaged replication forks. *Nat. Rev. Mol. Cell Biol.*, **3**, 859–870.

Mitchell, D. L. and Karentz, D. (1993) The induction and repair of DNA photodamage in the environment. *In* Environmental UV-B Photobiology, ed. by Young, A. R., Moan, J., Björn, L. O., and Nultsch, W., Plenum Press, New York, pp. 345–377.

Ordoñez, O. F., Flores, M. R., Dib, J. R., and Farías, M. E. (2009) Extremophile culture collection from Andean Lakes: Extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. *Microb Ecol*. DOI: 10.1007/s00248-009-9527-7 (in press).

Ponder, M. A., Gilmour, S. J., Bergholz, P. W., Mindock, C. A., Hollingsworth, R., Thomashow, M. F., Tiedje and J. M. (2005) Characterization of potential stress responses in ancient Siberian permafrost psychroactive bacteria. *FEMS Microbiol. Ecol.*, **53**, 103–115.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.,* **4**, 406–425.

Sarangi, A. and Krishnan, C. (2008) Comparison of in vitro Cr (VI) reduction by CFEs of chromate resistant bacteria isolated from chromate contaminated soil. *Biores. Technol.*, **99**, 4130–4137.

Tamura, K., Nei, M., and Kumar, S. (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA*., **101**, 11030–11035.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.,* **24**, 1596–1599.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*., **22**, 4673–4680.

Willems, A., Coopman, R., and Gillis, M. (2001) Comparison of sequence analysis of 16S–23S rDNA spacer regions, AFLP analysis and DNA-DNA hybridizations in *Bradyrhizobium*. *Int. J. Syst. Evol. Microbiol*., **51**, 623–632.

Wilson, C., Caton, T. M., Buchehhemein, M. A., Schneegurt, M. A., and Miller, R. V. (2004) DNA-repair potential of *Halomonas* spp from the salt plains microbial observatory of Oklahoma. *Microb. Ecol*., **48**, 541–549.

Winter, C., Moeseneder, M. M., and Herndl, G. J. (2001) Impact of UV-B radiation on bacterioplankton community composition. *Appl. Environ. Microbiol.,* **67**, 665–672.