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RESEARCH ARTICLE
INTERNATIONAL MICROBIOLOGY (2005) 8:235-242
www.im.microbios.org
INTERNATIONAL MICROBIOLOGY

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Seasonal microbial ribotype shifts in the sulfurous karstic lakes Cisó and Vilar, in northeastern Spain

Received 15 December 2004
Accepted 16 September 2005

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Summary. Spatio-temporal changes in two sulfurous lakes from the karstic area of Banyoles (Girona, Spain), holomictic lake Cisó and meromictic lake Vilar, were studied over one year. Samples were collected at different depths from the two lakes on the same days, during each of the four seasons, and several physico-chemical variables (temperature, light, pH, conductivity, sulfide, oxygen concentration, pigment concentrations, etc.) were measured. To fingerprint bacterial populations from each sample, DNA was extracted, bacterial 16S rRNA genes were amplified by PCR, and restriction fragment length polymorphism (RFLP) analyses of the total bacterial 16S rDNAs were performed. Each 16S rDNA pool was independently digested with three restriction endonucleases (*Alu*I, *Hin*fI, and *Rsa*I) and separated electrophoretically. More restriction fragments were obtained from the Lake Vilar samples than from the Lake Cisó samples. Moreover, intrasample and intersample differences were observed in each lake. RFLP patterns were compared by scoring similarities using the Jaccard coefficient and then building a multidimensional scaling (MDS) map from the resulting similarities matrix. In both lakes, results indicated that seasonality was mostly responsible for the observed fluctuations in the RFLP patterns, while the effect of stratification was less pronounced. [**Int Microbiol** 2005; 8(4):235-242]

Key words: Domain *Bacteria* · sulfurous lakes · seasonal shifts · 16S rRNA genes · restriction fragment length polymorphism (RFLP) · multidimensional scaling (MDS)

Introduction

Located in the northeastern Iberian Peninsula, lakes Cisó (42º 07′ 35″ N, 2º 45′ 05″ E) and Vilar (42º 07′ 10″ N, 2º 44′ 50″ E) are two neighboring sulfide-rich lakes belonging to the Banyoles karstic system (Girona, Spain) [1,18]. Although these lakes have the same climatic conditions and groundwater sources, their limnological characteristics are different [19]. Lake Cisó is holomictic, with a surface area of 650 m^2 and a maximum depth of 7 m [Gasol J (1988) PhD Thesis, Autonomous University of Barcelona], while Lake Vilar is meromictic, with a surface area of approximately 11,000 m^2 and a maximum depth of 12 m [1]. In lakes, bacteria make up most of the communities [16,40]. The microbial compositions of Lake Cisó and Lake Vilar differ markedly [7,8,13,19,39]. Both lakes are vertically stratified ecosystems due to physical and chemical gradients established along the water column (oxygen, sulfide, light, etc.). In these ecosystems, microbial communities are spatially distributed depending on the combination of these gradients, and their structures and species composition often change over time due to environmental fluctuations. Classical microbiological approaches allow the detection of only a very small propor-

tion of the bacterial assemblages [27,54]. More recent molecular methods have been described that provide important tools for studying changes in complex ecosystems [3,23,28, 30,31,38,45]. One of these techniques is the analysis of restriction fragment length polymorphisms (RFLP) of PCR-amplified bacterial 16S rRNA, which has turned out to be very useful to assess the diversity and space–time variations of microbial populations [12,14,24,29,33,34,42,46,55].

In previous work [41], we applied RFLP-PCR to study spatio-temporal changes of the predominant microbiota in microbial mats, which are benthonic multilayered microbial communities in which the photosynthetic layer expands for a few millimeters. Here we describe a similar study, carried out in multilayered microbial planktic communities, which are analogous to microbial mats except that they are much larger, ranging from a few centimeters to several meters [20]. In addition to studying shifts in the predominant indigenous ribotypes, the genetic structures of microbial assemblages that inhabit each lake were compared. The combination of molecular data and analysis of physico-chemical variables allows these genetic variations to be related to fluctuations in the environment [41].

Materials and methods

Sampling and procedures. Samples were collected in the spring (Sp) (May 22, 2000), summer (Su) (July 17, 2000), autumn (A) (October 9, 2000) and winter (W) (January 23, 2001) at different depths depending on the physico-chemical profiles measured in both lakes. Sampling was carried out using a Ruttner bottle on a small boat fixed at a set point coinciding with the maximal depth of each lake. Temperature, dissolved oxygen, light intensity, and conductivity were measured in the field using an oxymeter (Oxi 92, Crison Instruments, Barcelona, Spain), a luxmeter (Li-Cor model LI-189 Biosciences, Cambridge, UK,) and a conductivimeter (YSI model 33 S-T-C, Yellow Springs Instruments, Ohio, USA). For sulfide measurements, 10-ml subsamples were chemically fixed by adding zinc acetate to a final concentration of 0.1 M, and then analyzed spectrophotometrically in the laboratory using the methylene blue colorimetric method [52]. Water samples used for additional analysis were preserved at 4ºC for 3–4 h in the dark. In the laboratory, those samples were used for pH measurement (pH meter Orion model 420A, Orion, London, UK), protein [6] and pigment determinations (chlorophyll *a*, bacteriochlorophyll *a*, and bacteriochlorophyll $c + d + e$) [49,50], and DNA extraction.

DNA extraction. Two different protocols were used in duplicate. Firstly, 250 ml of water from each lake and each selected depth was centrifuged at 14,000 rpm for 20 min. The supernatants were discarded and the pellets were stored at –20ºC. Secondly, 100 ml of water was filtered (pore size, 0.22 µm; diameter, 47 mm) (Durapore membrane filters, Millipore, Billerica, Massachusetts, USA) and filters were stored at –20ºC until used. DNA from centrifuged and filtered lake samples was extracted as previously described [41]. When samples were collected from the deepest part of the lakes, crude DNA solutions always had a brownish color that interfered with spectrophotometric DNA detection and measurement. Thus, genomic DNA was quantified by electrophoresis and ethidium bromide staining [35,36]. To overcome the inhibition of the PCR reaction, a 10-fold dilution of these brownish crude extracts was used.

PCR amplification conditions. PCRs were prepared at 4ºC to avoid nonspecific priming. The total amount of DNA added to PCR mixtures was approximately 40 ng, except for samples from the deepest part of the lake. A 1500-bp DNA fragment from the 16S rRNA genes from the Domain *Bacteria* was amplified using primers ForB (5′ AGAGTTTGATCCTGGCTCAG 3′, corresponding to *E. coli* positions 8–27) and RevB (5′ GGTTACCTTGTTACGACTT 3′, corresponding to *E. coli* positions 1509–1491) (200 µM each) as previously described [41]. The presence of the expected PCR products was controlled by electrophoresis on 1% agarose gels in TAE (Tris-acetate-EDTA), followed by ethidium bromide staining (0.5 mg/ml) [44]. PCR reaction efficiencies were also checked by electrophoresis, and similar amounts of amplified products were used in restriction reactions.

16S rDNA RFLP. Restriction reactions were done for 1.5 h at 37ºC by incubating 45 ml of equal quantities of the amplified products with 30 U of each restriction enzyme (*Alu*I, *Hin*fI, or *Rsa*I) at 37ºC, according to the manufacturer's recommendation (Amersham, Life Science, Barcelona, Spain). The restriction fragments were analyzed by horizontal electrophoresis as described in [41]. As molecular mass standard, a 1-kb plus DNA ladder was included in the gels (Invitrogen). Restriction fragments shorter than 150 bp were disregarded to avoid confusion with potential dimer fragments of the primer [4].

Statistical data analysis. Statistical significances of the differences between the total number of restriction fragments obtained at different depths and different seasons were obtained by applying a Kruskal-Wallis analysis [http://www.basic.nwu.edu/statguidefiles/kruskal_wallis.html]. Also, significances of the differences between the average of the total number of restriction fragments (Â) obtained at different depths or different lakes were obtained using Student's *t* test.

Multidimensional scaling analysis (MDS) was applied as previously described [41]. Each restriction fragment profile was transformed into binary code depending on the presence (scored as 1) or absence (scored as 0) of a particular band. A tolerance value to compensate for misalignment of homologous fragments due to technical imperfections (0.5% for 150- to 900-bp fragments and 1% for 900- to 1500-bp fragments) was applied to each fragment. A similarity matrix for all pairwise combinations of RFLP profiles was constructed from the binary matrix, using the Jaccard coefficient as a measure of proximity [47]. The distance matrix was then used as data for the MDS analysis [28,53], available in the Systat package (Systat Software, London, UK), in which the data are presented in an Euclidean plane such that highly similar measurements are plotted close together.

Results

Physico-chemical variables. Lake Cisó was thermally stratified from spring to autumn, but completely mixed in winter. In contrast, Lake Vilar had a bottom layer of dense or highly mineralized water (monimolimnion) that never mixed.

With the exception of the incident light extinction, which decreased in depth from 120 µE cm^{-2} s^{-1} at 0 m to 0.1 µE cm^{-2} s^{-1} at 2 m, all the physico-chemical variables in Lake Cisó measured during the holomixis period were uniform in the entire water column, from the top to the bottom of the lake. During this period, the following values were measured: 1 ± 0.15 mg protein/l, 118.3 ± 19.3 mg chlorophyll *a* (Chl *a*)/l, 20.84 ± 2.6 mg bacteriochlorophyll *a* (Bchl *a*)/l, 8.43 ± 1.05 mg bacteriochlorophyll $c + d + e$ (Bchl c+d +e)/l, conductivity 1295 ± 19.9 mMhos/cm, 8.66 ± 0.43ºC, 0.68 ±

0.13 mg O_2/ml, sulfide concentration of 0.147 ± 0.01 mM, and pH of 7.37 ± 0.03. In the stratified period, a thermocline that separated the epilimnion from the hypolimnion was located at different depths depending on the season analyzed. The pH values ranged from 6.87 at 6 m in summer to 7.81 at 0–1 m in autumn. While conductivity and pH decreased with depth in summer, in the other thermal-stratification periods their values were similar throughout the lake. The maximum Chl *a*, Bchl *a*, and Bchl *c+d+e* concentrations were 761.3 mg/l at 2 m in spring, 55 mg/l at 6 m in summer, and 569 mg/l at 2 m in spring. Maximum protein values in spring, summer, and autumn were 0.93, 1.94, and 2.1 mg/l at 2, 6, and 7 m deep, respectively.

In winter, the extinction with depth of the incident light decreased less in Lake Vilar than in Lake Cisó, reaching a minimum value of 0.15 mE cm^{-2} s^{-1} at 8 m. Other variables measured, including protein concentration, Chl *a* concentration, conductivity, temperature, dissolved oxygen concentration, sulfide concentration, and pH, were similar from 0 to 8 m and within the following ranges: 0.518 ± 0.08 mg protein/l, 2.81 ± 1.71 mg Chl *a*/l, 913.78 ± 81.98 mMhos/cm, 8.99 ± 0.61ºC, 5.36 ± 2.05 mg O_2/ml, 6.32 10^{-4} ± 5.38 10^{-4} mM, and 7.72 ± 0.05. During the winter, Bchl *a* and Bchl *c+d+e* were detected only at low concentrations, at 4 and 9 m. In the monimolimnion, however, dissolved oxygen concentration decreased with depth and the highest values of conductivity and temperature were obtained from 10 m to the bottom. In the stratified period, the chemocline at the mixolimnion –monimolimnion interface occurred at different depths . The pH values ranged from 7.2 at 10 m in autumn to 8.1 at 0–1 m in spring. The highest Chl *a*, Bchl *a*, and Bchl *c+d+e* concentrations were found in summer: 31.66 mg/l at 6 m, 15.81 mg/l at 6 m, and 22.29 mg/l at 6 m, respectively. Maximum protein values in spring, summer, autumn, and winter were 1.28 mg/l at 10 m, 1.83 mg/l at 0 m, 0.92 mg/l at 11 m, and 0.69 mg/l at 9 m, respectively.

DNA extraction and bacterial 16S rRNA gene amplification. Genomic DNA extracted from standard strains and lake samples was approximately 23-kb in size. By PCR, the Domain *Bacteria* was detected as a 1500 bp fragment in samples from both lakes, Cisó and Vilar, at all sampling times and depths.

RFLP data. The restriction patterns from harvested samples were different in the two lakes, depending on the enzyme used and the depth analyzed. RFLP profiles obtained from filtered samples were identical to the profiles obtained from harvested samples (data not shown). Tables 1 and 2 show the numbers of fragments for each enzyme in Lake Cisó and Lake Vilar, respectively.

In Lake Cisó, the three enzymes gave consistent results for all seasons (Table 1). As expected, there were clear differences between the oxic profiles (spring 0–1 m, summer 0–2.5

Table 1. Number of fragments of 16S rDNA by restriction enzyme analysis in Lake Cisó

Season	Depth (m)	*Rsa*I	*Hinf*I	*Alu*I	Total
Spring	0	13	13	12	38
	1	7	14	12	33
	2	9	9	14	32
	3	10	9	14	33
	4, 5, 6	10	9	15	34
	7	8	6	5	19
Summer	0	10	10	15	35
	1, 1.25	9	8	14	35
	1.5	9	8	15	31
	2	9	8	12	29
	2.5	10	9	8	27
	3	14	11	12	39
	4	14	11	11	36
Autumn	0, 1, 2	8	13	14	35
	3	11	12	13	35
	4,5	11	11	13	35
	6	10	10	13	33
	7	10	10	12	32
Winter	0, 1, 2, 3, 4, 5, 6	14	9	11	34

Table 2. Number of fragments of 16S rDNA by restriction enzyme analysis in Lake Vilar

Season	Depth (m)	*Rsa*I	*Hinf*I	*Alu*I	Total
Spring	0, 1	15	12	14	41
	4	14	13	14	42
	5	14	14	14	39
	6	15	10	14	32
	8	11	10	11	38
	10	15	10	13	31
Summer	0, 1, 4, 5	10	10	11	31
	6	10	14	18	42
	6.5	11	16	14	41
	7, 7.5	11	16	13	40
	8	12	16	12	40
	10	12	17	12	41
Autumn	0, 1	11	12	17	40
	5	11	12	16	39
	6	11	11	16	10, 11
Winter	0, 1	11	14	19	44
	2, 3	11	14	18	43
	4	12	14	18	44
	7	13	14	18	45
	9	13	14	16	43

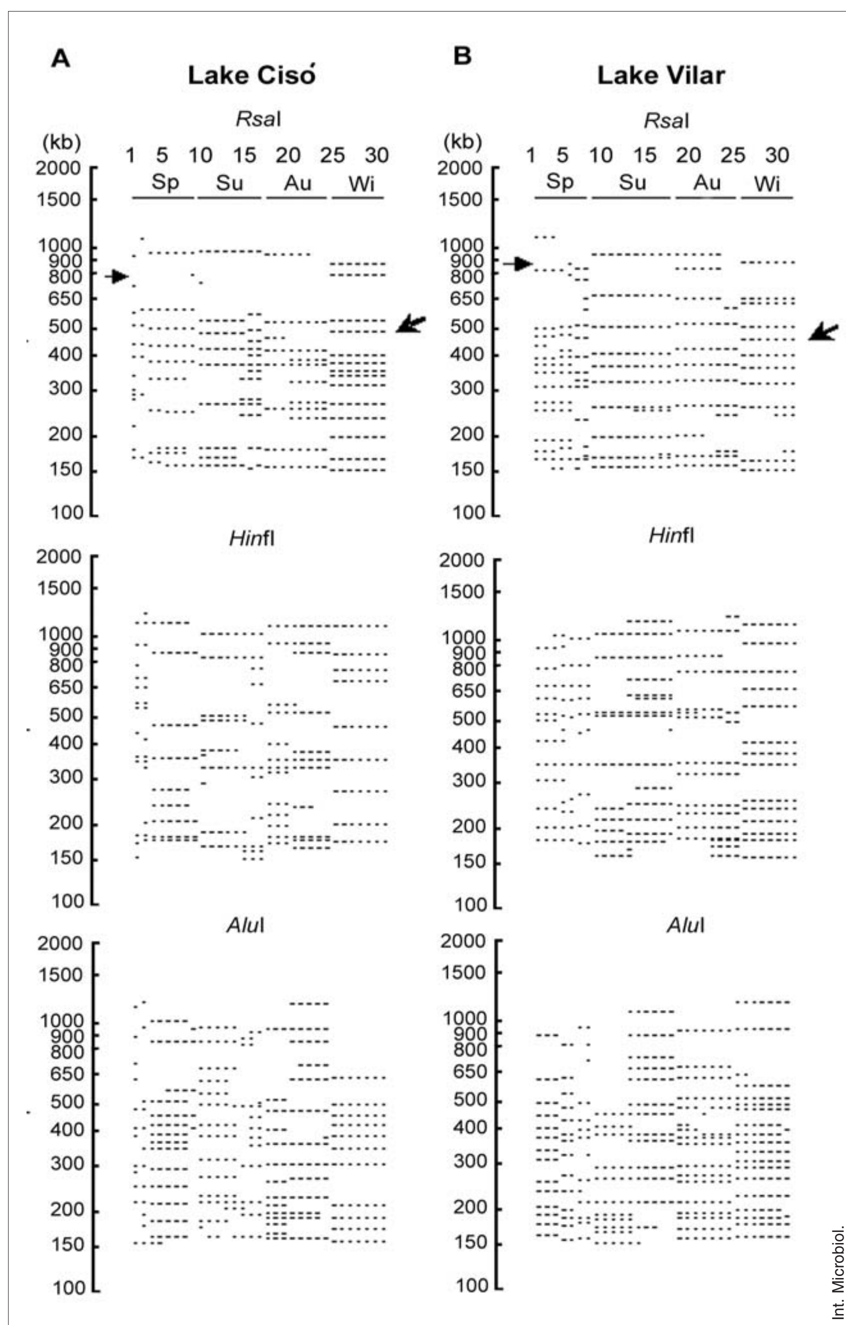

Fig. 1. Seasonal and depth distribution of bacterial 16S rDNARFLP patterns from samples harvested in spring (Sp), summer (Su), autumn (A) and winter (W) after digestions with *Rsa*I, *Hinf*I, and *Alu*I of samples from Lake Cisó and Lake Vilar. Lake Cisó: lanes 1–8: spring samples from 0, 1, 2, 3, 4, 5, 6, and 7 m; lanes 9–16: summer samples from 0, 1, 1.25, 1.5, 2, 2.5, 3 and 4 m; lanes 17–24: autumn samples from 0, 1, 2, 3, 4, 5, 6, and 7 m; lanes 25–32: winter samples from 0, 1, 2, 3, 4, 5, 6 and 7 m. Lake Vilar: lanes 1–7: spring samples from 0, 1, 4, 5, 6, 8, 10 m; lanes 8–17: summer samples from 0, 1, 4, 5, 6, 6.5, 7, 7.5, 8 and 10 m; lanes 18–25: autumn samples from 0, 1, 5, 6, 8, 9, 10, and 11 m; lanes 26–32: winter samples from 0, 1, 2, 3, 4, 7, and 9 m. Some fragments were common to all lines (e.g., bold arrow for W and S) whilst other were specific fragments (e.g., thin arrow for W and S).

m, and autumn 0–2 m) and the anoxic ones, in which sulfide concentrations were higher (spring 2–7 m, summer 3–4 m and autumn 3–7 m) (Fig. 1). However, in some anoxic subsamples the profiles were different in the deepest part of the lake (e.g., spring 7 m for *Hin*fI) while in others differences were observed depending on the enzyme used (autumn 3–7 m for *Alu*I). Moreover, some oxic subsamples differed in the uppermost layer of the lake (e.g., spring 0 m for *Rsa*I, *Alu*I, and *Hin*fI). RFLP patterns from the oxic–anoxic interfaces were similar in some cases to those from the anoxic subsamples (e.g., summer 2.5 m for *Hinf*I and summer 3 m for *Alu*I). During the holomixis period, the same RFLP patterns were obtained along the water column, following the vertical and uniform distribution of most of the physico-chemical variables analyzed. In spring, 38 restriction fragments were detected at the uppermost layer; 32–34 fragments at all intermediate depths; and only 19 fragments at the bottom layer, closest to the sediment. In summer, however, the number of

restriction fragments decreased with depth in the oxic part of the lake (summer 0–2.5 m) but increased in the anoxic one (summer 3–4 m). At the various depths, the number of fragments was similar in autumn (32–35 fragments) and equal in winter (34 fragments).

Table 2 shows that, in Lake Vilar, the largest number of restriction fragments was obtained in winter, and the numbers were similar at all depths assayed. The lowest number of fragments was obtained in spring and autumn; RFLPs were uniformly distributed in spring, but in autumn there were some differences between surface and deep samples. In summer, fewer restriction fragments were obtained in the oxic (i.e., summer 0.5 m) than in the anoxic zone of the lake (summer 6–10 m). The RFLP profiles of the samples from the oxic and the photic part of Lake Vilar in autumn, spring, and summer were more diverse than those of samples from the anoxic and aphotic zones. RFLP patterns from the oxic–anoxic interfaces were in some cases similar to those from either the anoxic subsamples (e.g., spring 6 m for *Hinf*I and spring 5 m for *Rsa*I) or the oxic ones (e.g., summer 6 m for *Rsa*I) (Fig. 1). In winter, RFLP profiles were similar throughout the entire water column.

More restriction fragments were obtained from Lake Vilar than from Lake Cisó. In both lakes, however, intrasample common fragments (e.g., bold arrow for sample W in Fig. 1) and specific depth fragments (e.g., thin arrow for sample S in Fig. 1) were present.

Data analysis. When the total number of fragments obtained at different depths and different seasons were compared, Kruskal-Wallis analysis showed a statistically significant seasonal variation (0.023, i.e., $p < 0.05$) in Lake Vilar, whereas no significant differences were found in Lake Cisó. Moreover, comparative analysis of the average number of fragments ($\hat{A}$) obtained at each depth by Student's t test showed a significant ($p < 0.001$) decrease in the average number of fragments at depths ≥7 m in both lakes. This test also showed a significant ($p < 0.001$) difference in the average number of fragments from Lake Vilar vs. Lake Cisó.

MDS analyses were carried out taking into account the similarity values of the cumulative number of *Rsa*I, *Alu*I, and *Hinf*I fragments. In both lakes, all depths associated with each lake and sampling event could be grouped together, thus forming four different major groups in the MDS maps (Fig. 2). Nevertheless, in Lake Cisó, there was a clear separation of the upper layers (SC 0–1 m; AC 0–2 m) from the deeper ones (SC 2–7 m; AC 3–7 m) in spring and autumn. Differences were observed under anoxic conditions, with good separation between samples from the deepest part of the lake (SC 7 m) and higher (SC 2–6 m). In winter, all subsamples were grouped together due to their identical RFLP patterns (Fig. 2A). In Lake Vilar, differences could only be observed between the oxic (SV 0–6 m, SuV 0–5 m, and AV 0–9 m) and oxic–anoxic interface parts of the lake, and the anoxic ones (SV 8–10 m, SuV 6–10 m, and AV 10–11 m), except for the winter sample, in which different patterns were detected depending on the presence of high- (WV 0–1 m) or low-intensity (WV 2–9 m) incident light (Fig. 2B).

In the MDS map, where highly related measurements are plotted close together, subsamples from spring were highly similar to those from winter and summer in Lake Cisó. The lowest similarity values were found between summer and winter subsamples in this lake. In Lake Vilar, however, each seasonal group was less dispersed, and low similarity values were found when subsamples from spring were compared with those from summer and winter. In this case, the highest similarity values were between summer and winter subsamples.

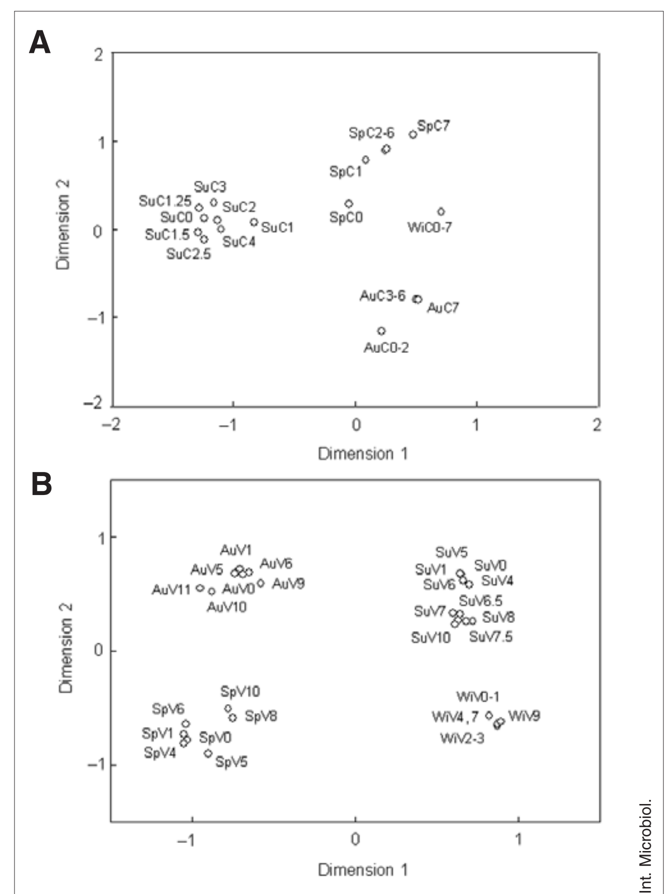

Fig. 2. Multidimensional scaling (MDS) maps obtained by the sum of fragments generated with *Rsa*I, *Hinf*I, and *Alu*I from samples harvested from Lake Cisó (**A**) and Lake Vilar (**B**). The results show the changes in the bacterial communities. The numbers outside the symbol (o) refer to subsamples.

Discussion

Vertical profiles for light, temperature, and oxygen concentration in both lakes were in accordance with previous studies [10,39, Mir J (1997) PhD Thesis, Autonomous University of Barcelona]. The sulfide concentration was low compared with previously reported data [7, Gasol J (1988) PhD Thesis]. Hydrogen sulfide is found at high concentrations from the bottom to the surface in Lake Cisó during the mixing period, whereas it is not detected in the upper layers in Lake Vilar during the same period, since it is oxidized at the interface between the anoxic and the oxic zones, as previously described [Mir J (1997) PhD Thesis].

The Domain *Bacteria* has been detected in both lakes at all sampling depths and times [1,9, Gasol J (1988) PhD Thesis]. Earlier studies showed that the bacterial populations of the two lakes differ. Members of the α-, β- and γ- Proteobacteria predominate in Lake Cisó, while the *Cytophaga-Flavobacterium-Bacteroides* phylum and Cyanobacteria are the dominant groups in Lake Vilar [8,9]. When RFLP patterns were analyzed, the total number of restriction fragments obtained during the four seasons in Lake Cisó was fairly consistent, whereas seasonal statistically significant differences were observed in Lake Vilar. Moreover, the numbers differed also between the two lakes (mean 40 per Lake Vilar and 33 per Lake Cisó), probably due to their different limnological origins and the conditions affecting the biotic and abiotic variables in these ecosystems [19,37]. Although it is feasible to obtain fingerprinting of the predominant ribotypes by RFLP analysis, it should be taken into account that the cell numbers of non-dominant bacterial populations might be too low to be detected by amplification and gel visualization of restriction fragments [42]. Also, the effects of inefficient PCR due to DNA structure or lack of primer complementarity cannot be ruled out [22,33,42,43].

In Lake Vilar, similar total numbers of restriction fragments were detected in the anoxic subsamples during summer, autumn, and spring. In the oxic layers, this number was higher in winter and lower in summer. The total numbers of restriction fragments increased with depth from 31 in the aerobic summer community (summer 0–5 m, Table 2) to 41 in the anaerobic one (summer 6–11 m, Table 2). This shift may be explained by population changes resulting from the different sulfide and oxygen concentrations in both parts of the lake. However, another explanation is the vertical motility responses of some phototrophic organisms to avoid death or severe damage from the high incident solar radiation in the upper layers, as was reported to be the case in microbial mats [11,26,41] and freshwater habitats [21].

In both lakes, intrasample RFLP differences were detected mainly between the oxic and anoxic parts of the lake. Pairs of oxic samples shared more bands than oxic–anoxic pairs, as described in previous studies carried out in stratified lakes using denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) [5,25]. When sharp gradients corresponding to the different physicochemical parameters are detected, the presence of common fragments in all depths of a sample may indicate that some ribotypes have adapted to a changing environment. As previously described in other aquatic ecosystems, those ribotypes probably represent microorganisms that have different and/or alternative metabolic pathways and which are able to migrate across the water column [15,17,32,48]. Nevertheless, and due to the conserved nature of ribosomal RNA, we cannot rule out that different microorganisms share identical restriction sites in their 16S rRNA genes and, consequently, fragments in RFLP patterns [42,52,55]. As was expected, no changes in the restriction patterns were observed in the holomixis period, according to the similar vertical profiles of the physico-chemical parameters. Thus, a few ribotypes seem to be dominant throughout the lake during this period. Moreover, specific fragments were also detected by RFLP analysis in almost all layers analyzed in the two lakes. This result indicated that some predominant ribotypes are present at specific depths and, consequently, in response to different environmental conditions [5,19,25].

When seasonal RFLP patterns were compared, the winter bacterial community in Lake Cisó shared more bands with the anoxic spring community than with the oxic one. This may be due to the appearance of new heterotrophic population members of the Domain *Bacteria* in the epilimnion [8]. Restriction patterns were compared by scoring similarities using the Jaccard coefficient and then building a MDS map from the resulting similarities matrix. The three enzymes gave consistent results, showing seasonal distributions instead of physiological ones. While previous studies showed that vertical stratification was the main factor determining the structure of populations in aquatic samples [2], our study shows that seasonal fluctuations are the most important ones in Lake Cisó and Lake Vilar. Similar results were obtained for benthic [30,41] and planktonic stratified ecosystems [39]. By principal component analysis of different biotic and abiotic variables, it was concluded that depth and seasonality determined the variability in Lake Cisó, while seasonality was the most important factor in Lake Banyoles [39, Gasol J (1988) PhD Thesis].

Our results are in agreement with previous studies carried out in benthic stratified ecosystems with seasonal community fluctuations [5,41]. Although a seasonal distribution evi-

dent in Lake Cisó and Lake Vilar, MDS maps were also capable of separating subsamples with different physico-chemical conditions resulting from the presence or absence of light and/or oxygen.

Acknowledgements. This work was supported by DGICYT grant PB97-0193 to Isabel Esteve (Department of Genetics and Microbiology, Autonomous University of Barcelona, Bellaterra, Spain). S. Ramírez-Moreno research was supported by a scholarship from the Spanish Ministry of Science and Technology. We thank the city hall of Banyoles for permission to collect samples. We are grateful to X. Munill, M. J. Bermúdez, I. Ferrera, and M. Llirós for their help in the sampling. We also thank A. Cabrera and A. Aguirre for their support in the statistical analyses.

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Cambios estacionales en los ribotipos microbianos en los lagos cársticos y sulfurosos Cisó y Vilar, en el nordeste de España

Resumen. Se estudió a lo largo de un año el cambio espacio-temporal que se produjo en dos lagos sulfurosos de la zona cárstica de Banyoles (Girona, España), el lago Cisó, holomíctico, y el Lago Vilar, meromíctico. Se tomaron muestras a diferentes profundidades en los dos lagos los mismos días durante las cuatro estaciones y se midieron algunas variables fisicoquímicas (temperatura, luz, pH, conductividad, sulfuro, concentraciones de oxígeno y de pigmentos, etc.). Para obtener la impronta genética de las poblaciones bacterianas de cada muestra, se extrajo el DNA, se amplificaron los genes del 16S rRNA mediante PCR y se analizó el polimorfismo en la longitud de los fragmentos de restricción (RFLP) del total de 16S rDNA bacteriano. Los diferentes conjuntos de 16S rDNA bacteriano fueron digeridos de manera independiente con tres endonucleasas de restricción (*Alu*I, *Hin*fI, y *Rsa*I) y separados por electroforesis. Se obtuvieron más fragmentos de restricción de las muestras del lago Vilar que del Cisó. Además, en cada lago se observaron también diferencias dentro de cada muestra y entre las diferentes muestras. Luego se compararon los patrones de RFLP puntuando las similitudes mediante el coeficiente Jaccard y la creación un mapa de escalamiento multidimensional (MDS) a partir de la matriz de similitudes resultante. Los resultados indicaron que la estacionalidad era la principal causa de las fluctuaciones observadas en los patrones de RFLP en ambos lagos, mientras que el efecto de la estratificación era menos pronunciado. [**Int Microbiol** 2005; 8(4):235-242]

Palabras clave: Dominio *Bacteria* · lagos sulfurosos · cambios estacionales · genes del 16S rRNA · polimorfismo en la longitud de los fragmentos de restricción (RFLP) · escalamiento multidimensional (MDS)

Mudanças estacionais nos ribotipos microbianos nos lagos cársticos e sulfurosos Cisó e Vilar, no nordeste da Espanha

Resumo. Ao longo de um ano se estudaram as mudanças espaço-temporários que se produziram em dois lagos sulfurosos da zona cárstica de Banyoles, o lago Cisó, holomíctico, e o Lago Vilar, meromíctico. Se tomaram amostras a diferentes profundidades nos dois lagos os mesmos dias durante as quatro estações e se mediram algumas volúveis físico-químicas (temperatura, luz, pH, condutividade, sulfureto, concentrações de oxigênio e de pigmentos, etc.). Para obter a estampagem em relevo genética das povoações bacterianas de cada amostra, se extraiu o DNA, se amplificaram os genes do 16S rRNA mediante PCR e se analisou o polimorfismo na longitude dos fragmentos de restrição (RFLP) do total de 16S rDNA bacteriano. Os diferentes conjuntos de 16S rDNA bacteriano foram digeridos de maneira independente com três endonucleasas de restrição (*Alu*I, *Hin*fI, e *Rsa*I) e separados por electroforesis. Se obtiveram mais fragmentos de restrição das amostras do lago Vilar que do Cisó. Além disso, em cada lago se observaram também diferenças dentro de cada amostra e entre as diferentes amostras. Depois se compararam os patrões de RFLP pontuando as similitudes mediante o coeficiente Jaccard e a criação um mapa de escalamiento multidimensional (MDS) a partir da matriz de similaridades resultante. Os resultados indicaram que a estacionalidade era a principal causa das oscilações observadas nos patrões de RFLP em ambos lagos, enquanto que o efeito da estratificação era menos pronunciado. [**Int Microbiol** 2005; 8(4):235-242]

Palavras chave: Dominio *Bacteria* · lagos sulfurosos · mudanças estacionais · genes do 16S rRNA · polimorfismo na longitude dos fragmentos de restrição (RFLP) · escalamiento multidimensional (MDS)