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Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting

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Abstract

Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments was used to compare surface bacterioplankton assemblages along the Catalan coast (NW Mediterranean). Samples from three coastal stations were compared with samples taken inside the Barcelona harbour and open sea samples taken during a cruise. The bacterial assemblage of each sample showed a characteristic and reproducible DGGE fingerprint. Between 17 and 35 bands were detected in each sample, and about 40% of the bands accounted for more than 80% of the band intensity in each sample. The presence of bands as well as their relative intensity was used to compare bacterial assemblages. Clear differences between the harbour samples and the coastal samples were evident during all periods. Marked temporal changes in the bacterial assemblages were detectable for the coastal sites, suggesting seasonal succession of coastal bacterioplankton. During each season, two stations presented a very similar bacterial composition (Barcelona and Masnou) whereas bacterial assemblages in Blanes were slightly different. These differences were consistent with the different hydrography of the area. Diversity indices calculated from DGGE fingerprints were relatively similar for all samples analysed, even though harbour samples were expected to present lower diversity values.

Keywords: Marine bacterial assemblage; Denaturing gradient gel electrophoresis; Fingerprinting; Diversity index

1. Introduction

Bacteria play significant roles in marine food webs and in most biogeochemical cycles in marine systems [1]. The taxonomic composition of bacterial assemblages and their temporal and spatial dynamics in the oceans are likely to be of major importance in determining the role of bacteria in marine biogeochemistry. At present, the time and space scales at which marine bacterial assemblages change their composition remain unknown. In offshore marine environments, depth in the water column seems to be the major factor determining bacterial composition [2–6]. Horizontal differences, on the other hand, seem to be relatively smaller, and it is remarkable how the same bacterial assemblage can be found at surface waters over large distances [2,6]. Coastal surface assemblages can be somewhat different from open sea assemblages [7], and could either be homogeneous over large shore distances, or they could be drastically affected by continental inputs or by the topography of the continental shelf. The extent of spatial variation of bacterial assemblages in relatively close coastal environments has not been studied.

The study of bacterial diversity in marine habitats has strongly advanced with the recent introduction of molecular techniques [8,9]. Different molecular methods have been used, and the choice of the method would be dependent on the number of samples to be processed within a reasonable period of time. In order to compare whole assemblages and assess temporal and spatial dynamics, a fingerprinting technique offers the best compromise. Denaturing gradient gel electrophoresis (DGGE), which resolves similarly sized DNA fragments (generated by PCR amplification) of different sequences, has already been successfully applied in field studies for such purposes [10–12]. In soil ecology, it has been used to profile microbial populations from microbial mats [13,14], and to study perturbation effects on agricultural soil assemblages [15]. In aquatic ecology, DGGE has been applied to study the

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vertical and temporal variation of prokaryote assemblages in freshwater [16,17] and marine systems [5,6]. In marine systems, it has also been applied to study the spatial distribution over large space scales [6,10]. These studies revealed that the DGGE technique is adequate to follow temporal and spatial dynamics of marine microbial assemblages. According to DGGE fingerprints, marine bacterial assemblages are generally dominated by few taxa (10–20), although many more are likely to be present at low abundances, and this agrees with the findings obtained with other techniques [3,18].

The aim of this work was to compare the bacterial composition of surface coastal assemblages in different sites along the Catalan coast (NW Mediterranean Sea). We wanted to test whether coastal bacterial assemblages behaved like offshore ones (being similar over long distances), or whether coastal features such as rivers or canyons caused heterogeneities in their compositions. Three sampling sites were chosen in order to reflect spatial differences potentially caused by topographical features (Fig. 1). A fourth sampling site, the Barcelona harbour, was chosen as a reference system, since the calm and protected harbour waters are expected to have distinct microbial assemblages. These four sites were sampled several times during the year. DGGE fingerprints obtained from selected samples were used to compare bacterial assemblages and calculate diversity indices.

2. Materials and methods

2.1. Study area and sampling

Surface seawater was collected from four stations along the Catalan coast (NW Mediterranean) from January to December 1998. Two sampling sites were located in Barcelona, one inside the harbour and the other on a pier on the beach, and were sampled directly from the shore. The other two sites, Masnou and Blanes, were 20 and 70 km north of Barcelona, respectively (Fig. 1), and were sampled at 400 m off the shoreline from a boat. Surface water temperature was measured in situ with a thermometer. Seawater was kept in 25-l plastic carboys for less than 2 h during transport until processing in the laboratory. Samples from the Hivern '99 cruise were collected on board R/V *García del Cid* in the Catalano-Balearic Sea during 3–14 March 1999. Surface water samples from two stations, one at the continental shelf near the coast (CC, Fig. 1) and the other in the open sea (CO, Fig. 1), were collected with Niskin bottles and processed in the same way as the coastal samples.

To collect microbial biomass, 1–15 l of seawater was filtered using a peristaltic pump through a 5 μm pore size Durapore filter (Millipore) and a 0.2 μm Sterivex filter (Durapore, Millipore) in succession. The Sterivex unit was filled with 1.8 ml of lysis buffer (40 mM EDTA, 50 mM Tris–HCl, 0.75 M sucrose) and stored at −70°C. Chlorophyll *a* concentrations were determined by measuring the fluorescence in acetone extracts with a Turner Designs fluorometer [19]. Briefly, 50−150 ml of seawater was filtered on GF/F filters (Whatman). The filters were extracted with acetone (90% v/v) in the dark at 4°C for 24 h before measuring the fluorescence. Subsamples for determination of prokaryote abundance were taken by fixing 1.2 ml of seawater with 1% paraformaldehyde+0.05% glutaraldehyde (final concentrations). Samples were allowed to sit in the dark for 10 min prior to transferring them to liquid nitrogen. Counting was performed in a FACScalibur flow cytometer (Becton-Dickinson) after staining cells with Syto13 (Molecular Probes) following the procedure outlined by Gasol and del Giorgio [20]. The coefficient of variation of prokaryote counts was always below 5%.

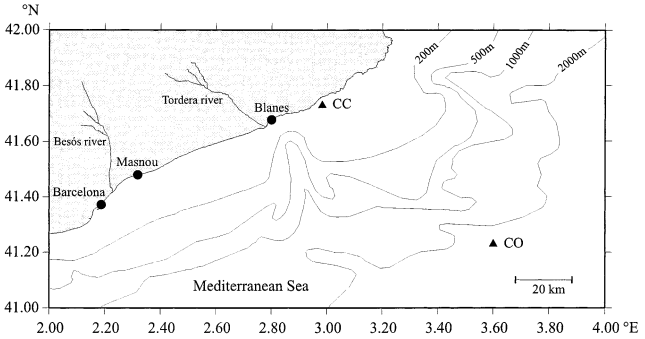

Fig. 1. Location of the stations sampled along the Catalan coast, NW Mediterranean: Barcelona (two sampling sites, harbour and coastal), Masnou and Blanes. Triangles show stations sampled during the Hivern '99 cruise. CC: coastal cruise sample; CO: open sea cruise sample.

2.2. DNA extraction and purification

Nucleic acid extraction began by adding lysozyme (1 mg ml^{-1}) to the Sterivex filter unit and incubating at 37°C for 45 min. Subsequently, proteinase K (0.2 mg ml^{-1}) and sodium dodecyl sulfate (SDS, 1%) were added, and the filter was incubated at 55°C for 1 h. The lysate was recovered from the filter and the filter was rinsed with 1 ml of lysis buffer. The pooled lysates were then extracted twice with an equal amount of phenol–chloroform–isoamyl alcohol (25:24:1, pH 8) and once with an equal amount of chloroform–isoamyl alcohol (24:1). The aqueous phase was spun down in a microconcentrator (Centricon-100, Millipore), washed with sterile TE buffer several times and reduced to a volume of 100–200 μl. The recovered DNA was quantified by a Hoechst dye fluorescence assay [21], and the quality of the DNA was checked by agarose gel electrophoresis. Extractions yielded 0.2–26.5 μg of DNA per liter of sample. Nucleic acid extracts were stored at −70°C.

2.3. PCR and DGGE

Microbial DNA (1 ng) was used as template for PCR

(polymerase chain reaction) amplification of bacterial 16S rDNA. The reactions (50 μl volume) contained 200 μM of each of the deoxynucleoside triphosphates, 0.3 μM of each of the primers, 1.5 mM $MgCl_2$, 1×PCR buffer and 1 U *Taq* DNA polymerase (Gibco BRL). We used the bacterial specific primer 358f, with a 40-bp GC clamp, and the universal primer 907r, which amplifies a 550-bp DNA fragment of bacterial 16S rDNA [11]. The PCR was performed with a Genius thermal cycler (Techne) using the following program: initial denaturation at 94°C for 5 min; 10 touchdown cycles of denaturation (at 94°C for 1 min), annealing (at 65–55°C for 1 min, decreasing 1°C each cycle) and extension (at 72°C for 3 min); 20 standard cycles of denaturation (at 94°C for 1 min), annealing (at 55°C for 1 min) and extension (at 72°C for 3 min), and a final extension at 72°C for 5 min. PCR products were verified and quantified by agarose gel electrophoresis with a standard in the gel (Low DNA Mass Ladder, Gibco BRL).

DGGE was performed with a DGGE-2000 system (CBS Scientific Company) as previously described [11]. A 6% polyacrylamide gel with a gradient of DNA-denaturant agent was cast by mixing solutions of 40% and 80% denaturant agent (100% denaturant agent is 7 M urea and 40% deionised formamide). 800 ng of PCR product was loaded for each sample and the gel was run at 100 V for 16 h at 60°C in 1×TAE buffer (40 mM Tris [pH 7.4], 20 mM sodium acetate, 1 mM EDTA). The gel was stained with the nucleic acid stain GelStar (FMC BioProducts) for 30 min, rinsed with 1×TAE buffer, removed from the glass plate to a UV-transparent gel scoop, and visualised with UV in the Fluor-S MultiImager (Bio-Rad) with the Multi-Analyst software (Bio-Rad). High-resolution images (1312×1034 pixels, 12-bit dynamic range) were saved as computer files (4.6 Mb).

2.4. Experiments to optimise PCR and DGGE

Several tests were performed to optimise the method and explore its reproducibility. In order to obtain sufficient amounts of PCR product and minimise the non-specific byproducts, we varied the template DNA concentration (0.01, 0.1, 1, and 10 ng DNA), the primer concentration (5, 10 and 20 pmol) and the *Taq* DNA polymerase brand (Gibco BRL or Ecotaq). The lowest template DNA and primer concentrations gave low PCR product yields, whereas the highest values tended to increase the byproducts. Both polymerases gave similar results. Next, we compared the DGGE fingerprint obtained by loading different amounts of the same PCR product, and observed that more bands were visible when more PCR product was loaded up to 800 ng. Larger amounts, however, produced a very high background and more intense bands tended to cover neighbouring lighter bands.

We then examined how reproducible the DGGE fingerprints were to describe complex assemblages. Variability in the fingerprints could potentially be introduced during biomass collection and nucleic acid extraction, during PCR, or during gel casting or electrophoresis. The last step was analysed first, since it was independent of the other two. The same PCR product from selected samples was loaded in separate gels and, although the exact position of bands could vary slightly, the DGGE pattern obtained was the same. The effect of non-reproducible PCR biases was addressed by running parallel PCR amplifications from the same sample, and the DGGE patterns obtained were identical. Finally, duplicate filters collected in Blanes were processed in parallel (independent nucleic acid extractions), and the replicates showed similar PCR amplification products and identical DGGE band patterns.

2.5. Quantitative analysis of DGGE fingerprints

Digitised DGGE images were analysed with the Diversity Database software (Bio-Rad). DGGE gels were composed of several lanes, each lane corresponding to a sample and including several bands at different positions with varying intensities. The software performs a density profile through each lane, detects the bands, and calculates the relative contribution of each band to the total band signal in the lane after applying a rolling disc as background subtraction. Then, the bands occupying the same position in the different lanes of the gel were identified. A matrix was constructed for all lanes, taking into account the presence or absence of individual bands, and the relative contribution of the band (in percentage) to the total intensity of the lane. This matrix was used to calculate a distance matrix using normalised Euclidean distances (root-mean-squared differences) with the software SYSTAT 5.2.1. Finally, a dendrogram comparing samples was obtained by UPGMA (unweighted pair-group method with arithmetic averages) in cluster analysis (SYSTAT).

In order to obtain direct descriptors of the diversity of bacterial assemblages, we calculated two widely used diversity indices, the Shannon index (H') and the Simpson index (D), as explained by Magurran [22] with the following formulae:

$$H' = -\sum_{i=1}^{i=n} p_i \ln p_i$$

$$D = \sum_{i=1}^{i=n} p_i^2$$

where n is the number of bands in the sample and p_i the relative intensity of the ith band. For convenience, the Simpson index is usually expressed as $1/D$.

3. Results

During 1998, a total of 60 samples over an entire sea-

sonal cycle were obtained from three stations along the Catalan coast and from the harbour of Barcelona (Fig. 1). One sample from each station, taken in winter (January), spring (April), summer (July) and autumn (November), was chosen to study the spatial differences of bacterial assemblages among the four sites. In addition, two surface water samples taken on a cruise in March 1999 off the Catalan coast (Fig. 1) were processed for comparison. The four sites exhibited similar surface water temperatures at each sampling date, ranging from 13°C in winter to 25°C in summer (Table 1). Prokaryote numbers and phytoplankton biomass (chlorophyll *a* values) were similar in the three coastal study sites, showing in general higher values in spring and summer than in autumn and winter (Table 1). Harbour samples showed significantly higher biomass of both phytoplankton and prokaryotes than the other stations over the entire year (ANOVA, $P = 0.000003$, $n = 14$) with extremely high values during summer.

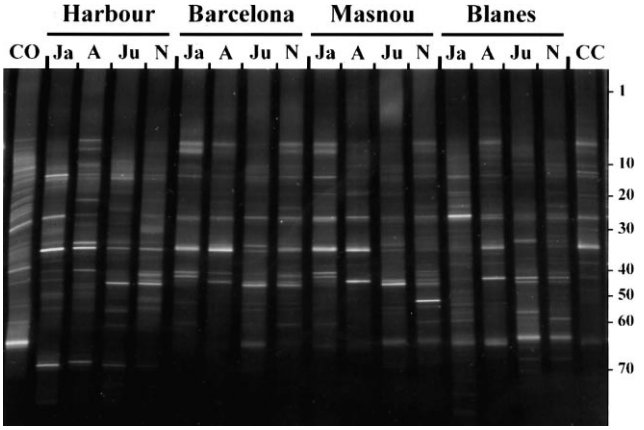

Fig. 2. DGGE gel showing PCR-amplified bacterial 16S rRNA genes for the 18 samples investigated. Ja: January samples; A: April samples; Ju: July samples; N: November samples; CC: coastal cruise sample; CO: open sea cruise sample. Numbers on the right show band positions.

We ran a DGGE gel including the 12 coastal samples, the four harbour samples and the two cruise samples (Fig. 2). The analysis of this gel resulted in a total of 453 detectable bands for the 18 samples processed. All bands detected by the software were above 0.2% relative intensity in a lane, which was the threshold applied. Several of the weaker bands could not be distinguished in Fig. 2, and were only detectable on the computer screen after zooming the image. The number of DGGE bands per sample varied between 17 and 35 (Table 1), being generally higher in Blanes samples (mean 28.3) compared to harbour (mean 26.8), Masnou (mean 23.5) and Barcelona samples (mean 21.8). No relationship was found between the number of bands detected and the abundance of prokaryotes or the chlorophyll *a* concentration in the sample. A few dominant bands accounted for most of the relative intensity in each lane (Fig. 2): in general, about 40% of the bands (6–14 bands) accounted for more than 80% of the total intensity per lane.

A schematic representation of the DGGE image shown in Fig. 2 is presented in Fig. 3. Comparison of the different lanes showed a total of 78 different band positions (Fig. 3). Seventeen bands were found at all sites at least once, indicating widespread phylotypes, whereas 31 bands were exclusively detected at only one sampling site, potentially indicating indigenous phylotypes. The remaining 27 bands appeared in two, three or four of the sites sampled. The Barcelona harbour was the site with the highest number of unique bands (15), followed by Blanes (9), Barcelona (4), and the cruise samples (3). In contrast, all bands from the Masnou samples were also present in other systems. Fig. 3 also shows that some bands appeared year-round, whereas

Table 1
Properties of samples used to investigate spatial differences among the study areas

Station	Sample	Date	Temperature (°C)	Chl *a* (μg l^{-1})	Prokaryote number (10^5 cells ml^{-1})	Number of bands	Shannon index (*H'*)	Simpson index (1/*D*)
Harbour	Jan	21 Jan 98	13.2	0.46	8.3	25	2.64	10.21
	Apr	27 Apr 98	14.5	6.28	16.7	28	2.81	12.93
	Jul	27 Jul 98	25.1	35.8	84.8	31	2.69	10.48
	Nov	6 Nov 98	18.0	0.82	10.1	23	2.90	12.60
Barcelona	Jan	21 Jan 98	13.8	0.35	1.9	20	2.54	8.32
	Apr	27 Apr 98	16.0	0.49	5.3	17	2.56	8.92
	Jul	27 Jul 98	21.6	1.27	5.8	24	2.63	9.50
	Nov	6 Nov 98	17.0	0.46	3.5	26	2.75	11.47
Masnou	Jan	22 Jan 98	13.9	0.31	2.2	24	2.72	10.25
	Apr	28 Apr 98	15.1	0.80	7.4	18	2.53	8.71
	Jul	28 Jul 98	24.9	1.33	6.9	24	2.60	8.60
	Nov	4 Nov 98	18.8	2.17	4.6	28	2.94	13.43
Blanes	Jan	27 Jan 98	13.5	0.26	2.5	35	2.94	11.36
	Apr	29 Apr 98	14.3	1.24	6.7	24	2.62	9.69
	Jul	29 Jul 98	25.5	0.13	4.2	23	2.79	11.37
	Nov	4 Nov 98	19.4	0.33	4.4	31	3.06	14.20
Cruise	CC	12 Mar 99	12.8	5.01	n.d.	20	2.46	7.51
	CO	11 Mar 99	13.5	1.41	n.d.	32	2.82	9.41

some other bands were specific to a particular season. Some bands dominated quantitatively (i.e. band 34 appeared in 17 of the 18 samples with a mean intensity of 15% of total intensity), whereas some bands were always very weak.

A statistical comparison of the bacterial assemblages of all samples analysed is shown as a dendrogram (Fig. 4). Coastal samples clustered mostly according to time of sampling, indicating that the temporal factor was more important than the spatial one in determining the bacterial composition. Several temporal clusters of samples were arbitrarily defined (Fig. 4). Barcelona and Masnou samples were very similar in the summer, winter and spring (Fig. 4), and only differed in autumn, when the Masnou sample was loosely associated with the summer cluster while the Barcelona sample was related to the winter cluster, suggesting an earlier shift to the winter bacterial assemblage in Barcelona than in Masnou. Blanes samples partially followed the division into seasonal clusters: the April sample belonged to the spring cluster, the July sample joined with the summer cluster, together with the November sample, whereas the January sample was loosely associated with the spring cluster. The greatest differences in clustering were found for the harbour samples. While the January and April samples were loosely associated with the winter cluster, the July and November samples did not show close relations to any of the other samples. Finally, the coastal cruise sample (CC) belonged to the winter cluster, with a close affiliation to the January samples of Barcelona and Masnou, and the offshore cruise sample (CO) belonged to the summer cluster and associated with the Blanes July and November samples (Fig. 4).

Shannon and Simpson diversity indices were calculated from the DGGE fingerprints in each sample (Table 1), and were used to compare the gross structure of bacterial assemblages among our samples (and not with other data sets). Both indices were rather stable in all samples (Table 1), with values between 2.46 and 3.06 (H'), and between 7.51 and 14.20 ($1/D$). Analysis of variance performed with all samples from the annual cycle showed that Blanes and harbour samples had similar diversity indices that were slightly higher than those for Barcelona and Masnou samples. Whereas the similarity between Barcelona and Masnou indices coincided with similar banding patterns, the similarity between Blanes and harbour indices resulted in fact from very different banding patterns.

4. Discussion

4.1. The use of DGGE to fingerprint bacterial assemblages

This study is based on the use of DGGE as a method to fingerprint marine bacterial assemblages. Prior to processing and comparing the samples, several tests were performed to optimise the method and explore its reproducibility under the conditions in our laboratory. These experiments demonstrated that the bacterial assemblage of each sample has a characteristic and reproducible DGGE fingerprint (including the presence and intensity of bands), which could be reliably used to compare different samples. The high reproducibility of DGGE and temperature gradient gel electrophoresis fingerprints has been reported in many other laboratories [6,10,17,23]. The primers used here also amplify plastidic 16S rRNA genes, and therefore some bands might derive from algal chloroplasts and not from bacteria. However, we believe this did not affect our results significantly because our samples were prefiltered through 5 μm (on average, this prefiltration removed 75% of chlorophyll *a*), and the number of algal cells was always several orders of magnitude below bacterial numbers.

The use of band intensity in our analyses deserves some discussion, since these values incorporate potential PCR biases (discussed later), and previous studies only used the binary information to construct similarity matrices [10,16] or dendrograms [15,23]. However, we demonstrated that band intensities were stable and reproducible in the same sample. The same band could be more or less intense in different samples indicating that this intensity was a characteristic of the sample. In addition, by using the band intensity our statistical analyses were less affected by weaker bands, leading to a higher reliability of the results. There was always a degree of subjectivity in deciding whether a very faint mark was a band, and including these very faint bands or not would significantly affect the dendrograms performed. The lesser impact of weaker bands in our results was shown in a simple exercise, in which the dendrogram and diversity indices were calculated excluding all bands that participated with less than 1% (instead of 0.2%) in the total intensity in the lane. On average, we removed 26% of the bands (2–11) in the 18 samples, but the topology of the dendrogram (not shown) was exactly the same as that shown in Fig. 4. Obviously, diversity indices decreased, but very slightly: on average the Shannon diversity index decreased by 5% and the Simpson diversity index decreased by 8% of the original values.

4.2. Comparison of marine bacterial assemblages

The number of bands in the DGGE fingerprint gives a rough estimate of the number of dominant phylotypes in the sample. Thus, very diverse bacterial assemblages, such as soils, presented so many bands that they could not be discriminated by DGGE [11]. On the other hand, a few bands were seen in simpler bacterial communities, such as microbial mats [13], or enrichment cultures [14]. Aquatic systems generally present an intermediate number of bacterial bands. In freshwater systems, 15–20 bands were detected in a meromictic lake [17] and 6–15 bands in a boreal

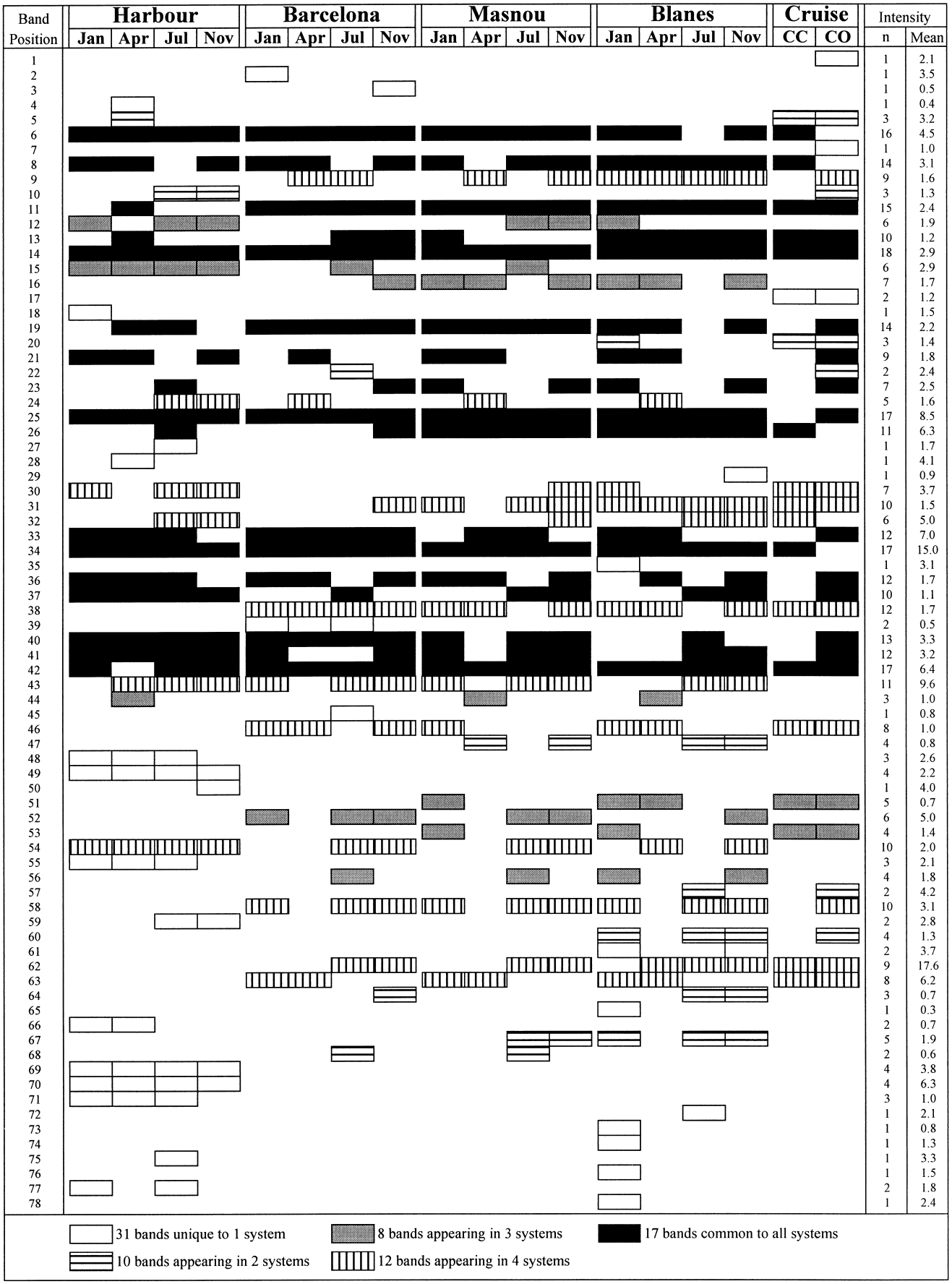
Band Position
Harbour
Barcelona
Masnou
Blanes
Cruise
Jan Apr Jul Nov
Jan Apr Jul Nov
Jan Apr Jul Nov
Jan Apr Jul Nov
CC CO
Intensity
n Mean
31 bands unique to 1 system
8 bands appearing in 3 systems
17 bands common to all systems
10 bands appearing in 2 systems
12 bands appearing in 4 systems

Fig. 3. Graphic representation of the DGGE gel shown in Fig. 2. Jan: January samples; Apr: April samples; Jul: July samples; Nov: November samples; CC: coastal cruise sample; CO: open sea cruise sample. Columns on the right give information about the quantitative importance of each band: the number of times it appears (*n*) and the mean relative intensity when it appears.
←

forest lake [16]. In marine systems, 15–30 bands were found in two Californian estuaries [10], 23–32 bands in Antarctic coastal waters [5], and around 15 bands in coastal and offshore samples in the Arabian Sea [6]. The samples analysed here, including coastal, harbour and offshore samples, presented 17–35 bands, consistent with previous results for marine bacterial assemblages.

All samples investigated presented similar numbers of bands and diversity indices, but clear differences in composition were apparent. As expected, the Barcelona harbour presented a distinct bacterial assemblage throughout the year (Fig. 4). This sampling site, situated in a protected part of the fishermen's harbour of Barcelona, is a highly contaminated and eutrophic environment, with a rather low water exchange with coastal waters. These distinctly different conditions from the other sites favour various phytoplankton blooms throughout the year [24], accompanied by high biomass and activity of the heterotrophic bacterioplankton. For the coastal sites, the temporal factor was very important, suggesting that coastal bacterioplankton could follow seasonal successions similar to that of coastal phytoplankton [24]. When the samples from each season were analysed separately, several spatial trends emerged. In the Catalano-Balearic Sea, there is a permanent front following the continental shelf break, separating the dense open sea waters of Atlantic origin from the less saline coastal waters with continental influence [25]. This front causes a current flowing southwest along the continental shelf, more or less parallel to the coast. Since the three sites are on the coastal side of this current, their bacterial assemblages were expected to be relatively similar. On the other hand, topographical features between the sites or local continental influences could alter this similarity. We demonstrated that Barcelona and Masnou samples exhibited very similar bacterial assemblages throughout the year, despite the different sampling strategies (shoreline in Barcelona, 400 m offshore in Masnou), the distance separating both stations (20 km), and the presence of the heavily contaminated river Besós flowing between the two sites.

Blanes, located 70 km north of Barcelona, is separated from Masnou by the Tordera river and by the Blanes submarine canyon (Fig. 1). It has been shown that canyons in this region can act as deflecting barriers to the coastal current and promote intrusions of offshore slope waters into the canyon, causing oceanic influence near the coast [26]. It has been noted that the distribution of zooplankton and microzooplankton can be influenced by the topography of the continental shelf in this zone [27,28]. The presence of the Blanes canyon could explain why the bacterial assemblages in Blanes were more different from those found in Barcelona and Masnou. The close grouping of the open sea cruise sample to the Blanes summer and autumn samples seems to be explained by these canyon-influenced local influxes of open sea waters into the shelf zone. The coastal cruise sample taken north of Blanes, however, is not directly affected by the current-modifying canyons and showed similar banding patterns to the coastal samples taken at Barcelona and Masnou at the corresponding time of the year.

4.3. Diversity indices for bacterial assemblages

It is very difficult to determine the number and relative abundance of bacterial species in a given system. It has been suggested that the use of species is not mandatory to calculate diversity [29], and some other units of classification have been proposed [30], among them the DGGE fingerprints [23,31]. There are many concerns in translating the diversity measured by DGGE to specific diversity. The number of bands could be an indicator of the number of abundant phylotypes in the sample. However, there could be heteroduplex formation during PCR [11], different sequences could appear at the same position in the gel,

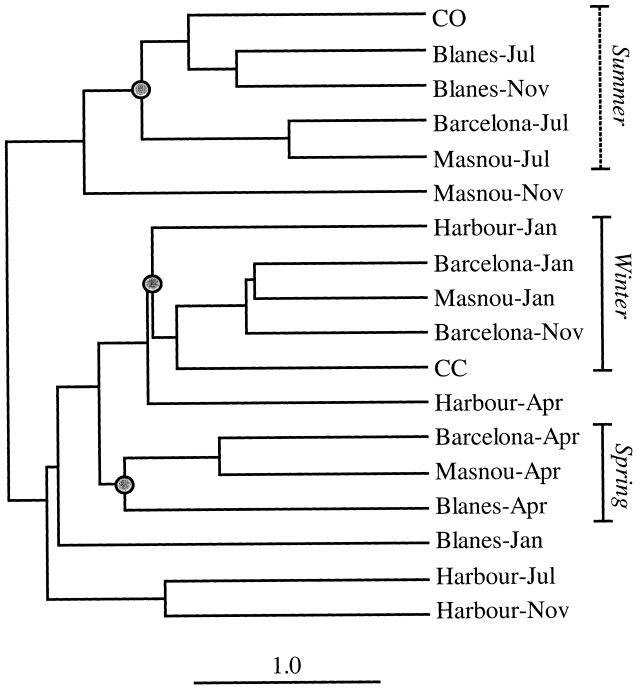

Fig. 4. UPGMA Euclidean distances dendrogram generated from the DGGE profiles of the 18 samples analysed. Jan: January samples; Apr: April samples; Jul: July samples; Nov: November samples; CC: coastal cruise sample; CO: open sea cruise sample. The samples are grouped into arbitrarily defined clusters to facilitate interpretation of the figure. Scale bar indicates Euclidean distances.

and different bands may correspond to different operons of the same organism [32]. The relative intensity of bands could be an indicator of the relative abundance of this phylotype in the sample. However, this value is dependent on the performance of the PCR, and PCR biases in which sequences are amplified preferentially have been described [33–35]. Despite these concerns, a recent study demonstrated that different 16S rRNA sequences amplified with the same efficiency [36], diversity indices calculated by three methods (morphotypes, DGGE and carotenoids) gave similar values [31], and some bacterial populations showed a good correlation between cell abundance and the intensity of its DGGE band [6,37]. We also found similar diversity indices when using different primer sets to the same samples (unpublished results). Therefore, although there are some inherent limitations, the available data [6,31,36,37] suggest that the diversity indices calculated from the DGGE fingerprints are reasonable estimates of the actual bacterial diversity in the samples. At the very least, they can be used to compare the structure of different bacterial assemblages when the same protocols have been used [23].

We assayed two indices with our samples, one more sensitive to richness (Shannon) and the other to evenness (Simpson). The values found were rather constant in all samples indicating that, although the particular taxonomic composition could vary among samples, the general structure of the bacterial assemblages was always the same (around 10 dominant bands). Shannon diversity indices, ranging between 2.5 and 3.0, fell within the range found in a variety of ecosystems for other organisms [22]. The similar diversity values between harbour and coastal samples was surprising. The biomass of phytoplankton and prokaryotes in the harbour was one order of magnitude higher than in the coastal sites. According to general ecological theory [38], a more eutrophic system is expected to be less diverse, but neither the number of bands nor any of the diversity indices calculated showed this trend.

In this work we have shown that the fingerprinting technique DGGE is a powerful tool to investigate natural microbial assemblages. It allows many samples to be processed in a less labour-intensive way than by cloning and sequencing. Our results suggest that bacterial assemblages on the Catalan coast follow seasonal dynamics and, as already reported in other studies [2,5,6], the same bacterial assemblage seems to occupy the same depth over large spatial scales. Coastal assemblages, because of the heterogeneity of their environment, can sometimes deviate from this trend, as in the case of Blanes samples at certain periods of the year, when local hydrographic conditions introduced sea water from offshore.

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