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ORIGINAL ARTICLE

Bacterial community structure of a coastal area in Kandalaksha Bay, White Sea, Russia: possible relation to tidal hydrodynamics

Chiara Pesciaroli · Belén Rodelas · Belén Juarez-Jiménez · Paolo Barghini · Massimiliano Fenice

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Abstract Kandalaksha Bay is an estuarine system located around the North Polar Circle in the White Sea (Russia). This peculiar environment, showing big sea level differences during tide cycles causing intense water mixing, is almost unknown concerning its microbial diversity. In this work, seawater bacterial communities, mainly obtained from a coastal area, were studied in order to gather information on their structure and most abundant populations. The study was carried out by cluster analysis of polymerase chain reaction–temperature-gradient gel electrophoresis (PCR-TGGE) fingerprinting of partial 16S-rRNA gene amplicons. Bacterial communities were strongly homogenized by tidal water mixing, especially on surface layers and close to the shore. Samples collected from the intertidal zone and the nearby sea surface grouped together with a high percentage of similarity, while those taken offshore at various depths showed evident differences. Multivariate analysis indicated depth as the most significant environmental parameter causing variations in the community structure. High levels of diversity were revealed by both the Simpson's index of diversity and the range-weighted richness index. The functional organization index suggested that the community was potentially able to preserve its functionality under stressing environmental perturbations. Sequencing of TGGE bands showed that most of the bacteria populations were evolutionarily close to α-proteobacteria. Some γ-proteobacteria and Actinobacteria were revealed too. This work represents the first major contribution to understanding bacterial diversity in Kandalaksha Bay.

Keywords Bacterial communities · PCR-TGGE · Biodiversity · Kandalaksha Bay · Intertidal zone

C. Pesciaroli · P. Barghini · M. Fenice (✉)
DEB, Department of Ecological and Biological Sciences, University of Tuscia, Largo Università snc, Viterbo 01100, Italy
e-mail: fenice@unitus.it

M. Fenice
Laboratory of Applied Marine Microbiology, Conisma, University of Tuscia, Largo Università snc, Viterbo 01100, Italy

B. Rodelas · B. Juarez-Jiménez
Department of Microbiology, Pharmacy Faculty, University of Granada, Campus of Cartuja, Granada 18071, Spain

B. Rodelas · B. Juarez-Jiménez
Water Research Institute, University of Granada, Edf. Fray Luis de Granada, C/Ramón y Cajal, 4, Granada 18071, Spain

Introduction

The marine environment is huge and comprises a myriad of different ecosystems with wide variety of organisms that are each adapted to unique conditions (Sumich and Morrissey 2004). In particular, microorganisms, due to their high level of diversity and adaptation, can colonize habitats generally inhospitable for any other organism (i.e. polar or deep seas and hydrothermal vents). In addition, since marine environmental conditions often change rapidly, microbial communities must express a strongly adaptive structure (Scheffer et al. 2001). A large amount of data is available on various marine peculiar habitats but, to date, a wide array of marine environments is still unknown or scarcely investigated from a microbiological point of view. Moreover, for many microbiological studies, detailed information on the overall community organization is frequently missing (Fuhrman et al. 2006; Allison and Martiny 2008).

The White Sea is an enclosed basin situated in the Arctic Circle, but it is considered a sub-extreme environment (Pantyulin 2003). Kandalaksha Bay (KB) is an estuarine system showing significant sea level differences during tide cycles causing intense water mixing (Melnikov et al. 2003; Savvichev et al. 2003). However, in some KB areas, such as the "Velikaja Salma" bay, both stratified and mixed regions

are present (Pantyulin 2003). The seasonal extreme runoff of freshwater, due to the various rivers and strong precipitations, contributes to the very peculiar hydrodynamics of the bay (Howland et al. 1999; Dolotov et al. 2005). The intertidal zone (littoral) is particularly interesting because all biogeochemical processes are most evident. This is especially true if tidal phenomena are very pronounced, as in KB where the littoral zone is around 30–40 m. Organisms in this zone need to be adaptable to variable environmental conditions in which factors, such as water availability, temperature, and salinity, change frequently (Savvichev et al. 2004; Kravchishina et al. 2008). Here, microbial communities must have a functional organization that is apt to counteract the effects of sudden stressing conditions.

The knowledge of microbial diversity and its role in nature is often inadequate, mainly because traditional techniques, such as the use of microscopy and cultivation methods, yield only limited information on community structure and ecosystem functionality (Hugenholtz et al. 1998; Webster et al. 2001; Lewis 2007). As for other environments, the large majority of marine microorganisms cannot be isolated in pure cultures or fail to grow in laboratory conditions (Ward and O'Mullan 2002; Lewis 2007; Zhao et al. 2008). Thus, to obtain an overview of presence, diversity and the role of microorganisms in the ecosystem, culture-independent methods are much more adequate (Wagner et al. 1993; Felske et al. 1999; Spring et al. 2000).

Temperature-gradient gel electrophoresis (TGGE) is a cheap and rather simple fingerprinting technique that is particularly useful for the analysis of microbial communities in environmental samples (Muyzer 1999). Using taxonomic targets, TGGE can reveal the community genetic diversity allowing the phylogenetic affiliation of its most abundant components (Muyzer and Ramsing 1995; Ranjard et al. 2000; Gómez-Silván et al. 2010). Moreover, it could supply information on community structure and dynamics (Bent and Forney 2008). This technique, or the quite similar DGGE, has been successfully applied to the study of microbial communities in extreme or contaminated environments (Federici et al. 2012; Tocchi et al. 2012; Maza-Marquez et al. 2013; Reboleiro-Rivas et al. 2013).

Only a limited number of studies deal with White Sea microorganisms and even less regard KB. Most of the studies were carried out with cultivation-dependent methods, thus, the available information is very limited (Savvichev et al. 2003, 2004; Kravchishina et al. 2008). To the best of our knowledge, no prior studies using culture-independents methods have been conducted on microbial communities from this area and no comparison of different sea sectors had been carried out.

The aim of our investigation concerning the Kandalaksha Bay bacteria was to provide the first detailed report concerning the overall presence of different microbial groups in this peculiar environment and their organization in functional communities. In this work we performed a polymerase chain reaction (PCR)-TGGE fingerprinting comparative study of the bacterial communities in water samples collected from an intertidal zone and the nearby coastal area. Samples from other open sea locations and different depths were considered too. In addition, to understand the influence of various environmental parameters on the bacterial community structure, a multivariate analysis has been performed. The structure of bacterial communities is also discussed in relation to tidal hydrodynamics.

Materials and methods

Collection of samples

Seawater was collected in various KB areas as previously reported (Pesciaroli et al. 2012). Briefly, the main sampling sites were an intertidal zone and the nearby sea surface located on the Cape Kindo peninsula in "Velikaja Salma" Bay (Fig. 1). Samples were collected consecutively for 8 days at the minimum tide level from an intertidal pool (samples from P1 to P8) and from the adjacent open seawater surface (samples from M1 to M8). The site location was chosen for its connection with the whole bay water circulation caused by direct exposure to the intense tidal flow. Samples were also collected offshore at different depths and locations (samples from N1 to N4). The physico-chemical characteristics of sample sites are summarized in Table 1. After sampling, 250 mL of water were vacuum-filtered on sterile membranes (0.22 μm, Millipore, USA). Dry membranes were maintained at 4 °C in sterile tubes. Sterile silica gel was added to keep the membrane dry prior to DNA extraction.

DNA extraction

Total DNA was extracted from the membrane filters as follows. Each membrane was suspended in ca. 2 mL of sterile water and triturated grossly with a sterile pipette tip; tubes were then stirred by vortex (IKA, Germany) in order to re-suspend the DNA. The suspension was transferred to a clean microcentrifuge tube and used for DNA extraction by the MasterPureTM purification Kit (Epicentre® Biotechnologies, USA) according to manufacturer's instructions.

PCR amplification of partial 16S rRNA genes from bacteria communities

A nested PCR approach was selected for specific amplification of the V3 hypervariable region of the 16S rRNA gene of

Fig. 1 Map of sampling sites within Kandalaksha Bay, White Sea, Russia. **A** Cape Kindo peninsula samples P, M, N1 and N3: at ca. 66°32′54″N, 33°08′30″ E. **B** samples N2 and N4: at ca. 66°29′21″N, 33°38′20″E

the bacteria, as previously described (Reboleiro-Rivas et al. 2013). DNA (2–5 ng), extracted as described above, was used as a template for a first PCR carried out using the universal 16S-rRNA gene primers fD1 and rD1 (Weisburg et al. 1991). Subsequently, nested PCR was performed using as a template 1 μL of the first PCR product and the V3 region universal primers given by Muyzer et al. (1993): GC-341f (CGCCCG CCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG ATCCCTACGGGAGGCAGCAG) and 534r (ATTACCGC GGCTGCTGG). Conditions for each PCR reaction were as previously described (Reboleiro-Rivas et al. 2013).

TGGE fingerprinting and analysis

Experiments were done on a TGGE Maxi system (Whatman-Biometra, GmbH, Germany). Denaturing gels, comprised of 6 % polyacrylamide (37.5:1 acrylamide:bisacrylamide), 20 % formamide, 2 % glycerol and 8 M urea, were run with 2 × TAE (Tris–acetate–EDTA) buffer at 125 V for 18 h. The optimal temperature gradient for efficient band separation was 43–53 °C. Gel bands were visualized by silver staining using the Gel Code Silver Staining kit (Pierce, Thermo Fisher Scientific, Rockford, IL,USA) following the manufacturer's

Table 1 Sampling dates and physico-chemical characteristics of sample sites in Kandalaksha Bay

Date	Sample	Air temp. (°C)	Water temp. (°C)	DO (mg L^{-1})	Salinity (‰)	Rainfall (mm h^{-1})	Weather
05/09/08	P1	11.9	10.1	9.7	23.0	6.2	Heavy rain, no wind
	M1	11.9	10.1	9.8	23.7	6.2	
06/09/08	P2	13.0	11.3	9.6	23.0	0	Sunny, moderate wind from W
	M2	13.0	10.8	9.5	24.1	0	
07/09/08	P3	11.7	10.3	9.7	23.8	0	Sunny, no wind
	M3	11.7	10.2	9.7	23.3	0	
08/09/08	P4	10.7	9.4	9.9	23.4	2.3	Cloudy after rain, wind from N
	M4	10.7	9.1	9.9	23.5	2.3	
09/09/08	P5	8.9	8.7	10.0	24.9	0	Partially cloudy, no wind
	M5	8.9	8.4	10.0	24.4	0	
10/09/08	P6	8.8	8.4	10.0	24.4	0	Partially cloudy, no wind
	M6	8.8	8.7	10.1	24.2	0	
11/09/08	P7	8.5	7.6	10.6	23.7	0	Cloudy, no wind
	M7	8.5	7.5	10.6	24.0	0	
12/09/08	P8	6.8	6.4	10.7	23.4	0	Sunny, no wind
	M8	6.8	6.9	10.5	24.6	0	
06/09/08	N1	13.5	8.5	9.9	23.5	0	Sunny, moderate wind from W
	N3	10.5	6.6	10.6	24.2	2.3	
08/09/08	N2	13	8.0	10.2	24.0	0	Cloudy after rain, wind from N
	N4	13	0	10.9	25.4	0	

Sampling sites: *P* intertidal zone pool, *M* water surface near the intertidal zone pool, *N1* water collected by scuba divers at −2.5 m, *N3* water collected by scuba divers at −15.0 m, *N2* water collected with boats at 0.0 m, *N4* water collected with boats at −70 m

indications. In order to reduce staining of the gel background, the stabilization step was omitted. A six-species marker was included to aid normalization of the gel images (Vílchez et al. 2007).

TGGE band patterns were normalized, compared, and clustered using the Gel Compar II image analysis software (version 5.102, Applied Maths, Belgium). Bands were automatically detected and matched, and further corrections were applied manually. For cluster analysis, the TGGE profiles were compared using a band assignment-independent method based on the Pearson's product correlation coefficient taking into consideration both band position and intensity. Profiles were also compared by the Dice band-based similarity coefficient: band-matching (band assignment). Dendrograms, relating band pattern similarities, were automatically calculated with UPGMA algorithms (Unweighted Pair Group Method with Arithmetic Mean). The significance of UPGMA clustering was estimated by calculating the cophenetic correlation coefficients (Sokal and Rohlf 1962).

From TGGE fingerprints, several indices were calculated to analyze population biodiversity. Band number and intensity were used in each profile as representations of the number and relative abundance of the different species in each sample. To evaluate sample diversity, Simpson's index of diversity (*Sd*) was calculated as follows: $1\text{-}D = 1\text{-}\Sigma(p_i)^2$, where p is the proportion of an individual band intensity relative to the sum of all band intensities, and the value of 1-D ranges between 0 and 1 (Simpson 1949). The higher the value of 1-D becomes, the higher the diversity will be, so diversity increases as richness increases. Additionally, the range-weighted richness index (*Rr*) was calculated, based on the total number of bands in each TGGE pattern (N) and the temperature gradient (°C) comprised between the first and last band of each pattern (Tg) (Marzorati et al. 2008). The resulting values were divided by 100 (Gómez-Silván et al. 2010) to keep an order of magnitude analogous to that of the *Rr* index as originally described for DGGE by Marzorati et al (2008). To render a graphical representation of the evenness of the bacterial communities in the different sampling sites, Pareto-Lorenz distribution curves were drawn as proposed for DGGE by Marzorati et al. (2008). The bands in each TGGE lane were ranked from high to low based on intensity levels. The cumulative normalized band intensities for each TGGE lane were plotted against their respective cumulative normalized number of bands. The curves were numerically interpreted by the Functional organization index (*Fo*), given by the horizontal y-axis projection on the intercept with the vertical 20 % x-axis line (Marzorati et al. 2008). The calculation of the *Fo* (%) allows the evaluation of functional redundancy of the microbial communities analyzed by fingerprinting methods (Marzorati et al. 2008).

DNA sequencing of TGGE-isolated bands, phylogenetic and molecular evolutionary analyses

Portions of prominent bands were picked up with sterile pipette tips from silver stained gels, placed in 10 μL of filtered (0.22 μm) and autoclaved distilled water, and directly used for reamplification with appropriate primers. PCR products were purified and sequenced by an ABI PRISM 3100 Avant genetic analyzer. Sequences were analyzed online by the European Bioinformatics Institute biocomputing tools (http://www.ebi.ac.uk). The BLASTn program (Altschul et al. 1997) was used for preliminary sequence similarity analysis. The ClustalX version 2.0.3 software (Jeanmougin et al. 1998) was used for sequence alignment. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Kumar et al. 2008). A p-distance-based evolutionary tree was inferred using the neighbor-joining and/or the maximum likelihood algorithms (Saitou and Nei 1987). The bootstrap test was conducted to infer the reliability of branch order (Felsenstein 1985), with a round of 1,000 reassembling. Bootstrap values below 50 % are not shown in the tree.

Statistical multivariate analysis (redundancy analysis, RDA)

TGGE band patterns generated by GelCompar II were converted to a binary matrix by scoring band presence, "1", or absence, "0". This matrix was preliminary evaluated, without transformation, by detrended correspondence analysis (DCA) showing a linear, rather than unimodal response to the environment (lengths of gradient ≤3) (Lepš and Šmilauer 2003). Accordingly, redundancy analysis (RDA) was chosen to reveal relationships between the structure of KB communities and a set of environmental variables such as water temperature, dissolved oxygen (DO), salinity, depth, and precipitations. All environmental variables were transformed to log (X+1). The Monte Carlo permutation test was used to assess the statistical significance of ordination axes. All the multivariate statistics were computed using the "Canoco for Windows" v. 4.5 software (ScientiaPro, Budapest, Hungary).

Results

Cluster analysis with Pearson and Dice coefficients

Pearson coefficient clustering was calculated on the densitometric curves obtained for each sample. Thus, samples similarity refers to both band positions and intensities recorded in each profile. To test TGGE fingerprint reproducibility, duplicate PCR reactions of the same sample were run on the same gel and analyzed. Comparison of samples N3 and N4 in duplicate is displayed in Fig. 2a, showing that the intrinsic method variability was below 15 %. The results evidenced

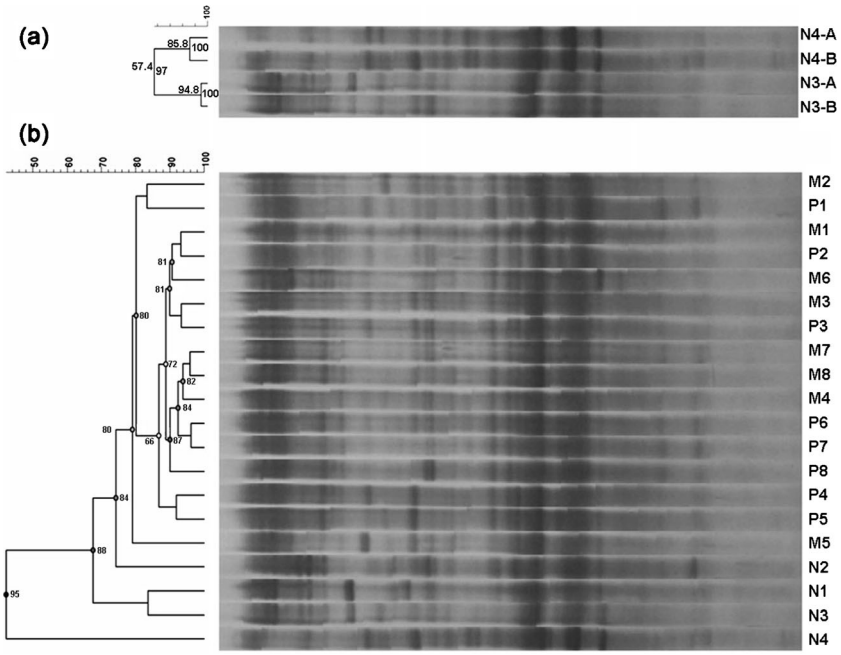

Fig. 2 Kandalaksha Bay total bacteria community: Pearson coefficient-based analysis of TGGE fingerprint band patterns. **a** Method reproducibility: duplicate TGGE run of samples N3 and N4. **b** Comparison of TGGE fingerprint band patterns from the various samples (P1 to P8 = intertidal pool samples, M1 to M8 = water surface nearby the intertidal zone pool samples, N1 to N4 = open-sea samples taken at various depths)

diversity in the community structure of different sampling sites (Fig. 2b). A big cluster included all the samples taken in the intertidal pool and the nearby sea surface (lanes from P1 to M8). Fingerprints of these samples grouped together, showing rather high similarity (80 %). The only sample that clustered slightly away was M5. On the contrary, samples taken in open sea, at different depths, clearly clustered away. This was particularly evident for the −70 m sample (N4), which branched at only 40 % of similarity. Among the open-sea profiles there was certain variability, but the fingerprints of samples taken at −2.5 m and −15 m (N1 and N3) were quite similar (ca. 80 %). Finally, the sample from the open-sea surface (N2) was rather similar to those from the coastal area (P and M samples) clustering at ca. 75 % of similarity.

Similarity among samples based on band presence/absence in the profiles, without considering band intensity, was calculated by the Dice coefficient. In this case, reproducibility was higher than that obtained by Pearson's being the intrinsic method variability lower than 5 % (Fig. 3a). Again, fingerprint grouping was clearly related to the sampling sites (Fig. 3b). The similarity of the P and M samples was even higher (ca. 85 %) than that obtained by the Pearson coefficient, showing the presence of almost the same bands in all samples. Among them, only P3 showed some differences. Open-sea samples grouped in two different clusters, both showing low similarity with P and M samples (<75 %). Differently from the results obtained by the Pearson coefficient, N2 clustered together with N1 with high similarity (>85 %) and it was not similar to P and M. In addition, differences between N3 and N4 were much lower (similarity ca. 74 %), and they clustered more away from all others samples (69 %).

Number of bands, diversity indices, and functional organization

Image analysis revealed a total of 70 different band classes (Fig. 3b). The average number of bands (Table 2) per sample in the coastal area (P and M) was ca. 26, with a minimum of 23 for P1 and M5 and a maximum of 29 for P3 and M8. As for the open sea, the average number of bands was 30. The minimum number of bands was detected in N3 (27 bands), and the maximum in N2 and N4 (33 bands).

Results of *Sd*, taking into account both richness and evenness, are reported in Table 2. All samples showed very high levels of diversity, but no evident differences were recorded in relation to the sampling site or depth.

Range-weighted richness index (*Rr*) also providing an estimation of microbial diversity, reaching high values in all the sampling sites (Table 2). This was especially evident for N samples (average *Rr* = ca. 69). Figure 4 reports *Fo* values calculated for the various sampling sites. P and M samples (Fig. 4a and b) reached, on average, ca. 62 %, while *Fo* of the N samples (Fig. 4c) ranged from ca. 50 to ca. 65 %. *Fo* defines the community ability to be organized in adequate distribution of dominant and resilient microorganisms consenting to counteract the effect of sudden stressing conditions (Marzorati et al. 2008). The community functionality (TGGE profiles) could be graphically represented by Pareto–Lorenz evenness curves. *Fo* values are expressed as a percentage. Perfect evenness, represented by the 45° diagonal, means that all the community species have the same number of individuals. Low *Fo* values indicated that the community is scarcely functionally specialized because the number of individuals in each species is quite similar and a relatively long lag phase could be

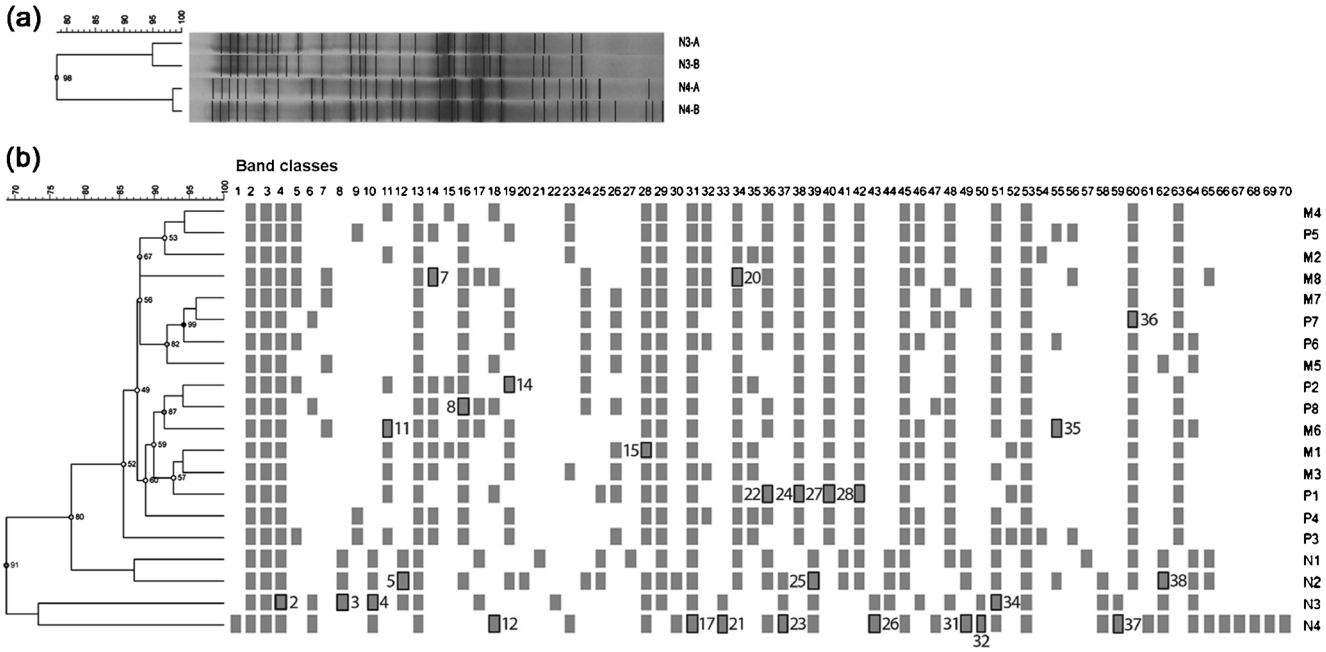

Fig. 3 Kandalaksha Bay total bacteria community: Dice coefficient-based analysis of TGGE fingerprint band patterns. **a** Method reproducibility: duplicate TGGE run of samples N3 and N4. **b** Comparison of the band presence/absence matrix, generated by the software GelCompar II, from the TGGE fingerprint band patterns (P1 to P8 = intertidal pool samples, M1 to M8 = water surface near the intertidal zone pool samples, N1 to N4 = open-sea samples taken at various depths): each rectangle represents a band; each column represents a class of bands, *black surrounded rectangles* followed by a number representing excised and re-amplified bands

necessary after a stress exposure. By contrast, high values of *Fo* represent a very specialized community with a small amount of dominant species.

Statistical multivariate analysis (redundancy analysis, RDA)

RDA showed that, according to the results of the Monte Carlo permutation test, the most significant factor explaining the variation of KB fingerprints was depth ($p<0.0020$) (Fig. 5). A strong correlation of the sample profiles with depth was found, as this factor was mainly correlated to the 1st ordination axis ($r=0.94$) which described 28.0 % of the total sample variance of species data (samples) and 55.4 % of the variance of the sample-environment relationship. Lower, although significant, correlations were found for DO and temperature and no significant correlation was found for either salinity or rainfalls.

Table 2 Average number of bands, diversity, and range-weighted richness indexes calculated for different sampling sites

Sample	N°	*Sd*	*Rr*
P1-P8	25.9±2.0	0.89±0.02	45.9±2.0
M1-M8	25.9±1.7	0.88±0.02	46.0±6.5
N1-N4	30.0±3.0*	0.92±0.02	55.5±5.3 *

P intertidal pool samples, *M* water surface near the intertidal zone pool samples, *N* open-sea samples taken at various depths. *N*° average number of bands, *Sd* Simpson's diversity index, *R* range-weighted richness indexes. Values represent means ± standard deviation. Values followed by asterisks indicate significant differences from the other values in the same column (Tuckey test, $p<0.01$)

Taxonomical affiliation and phylogenetic study

To obtain a detailed overview of the predominant species of the KB bacterial community, prominent TGGE bands were re-amplified and sequenced to perform a phylogenetic study (Fig. 3b). A total of 27 bands were successfully re-amplified and sequenced. After comparison with the EMBL sequences database, a phylogenetic tree was generated to visualize evolutionary relationships between sequences of KB samples and those of the closest affiliated relatives (Fig. 6). Most of the sequences were related to marine α-proteobacteria (16 out of 27, ca. 59 %) belonging to the order *Rhodobacterales* and mainly grouped in the periphery of the genus *Roseobacter*. A couple of sequences of this cluster (bands 25 and 26) were related to an unidentified marine sponge bacterium and an Arctic sea ice bacterium clone, respectively. Sequence 36 can be affiliated to *Ruegeria* sp. (*Rhodobacterales*). The second important cluster gathered all γ-proteobacteria and included seven sequences highly similar to the moderate halophilic bacterium *Cobetia marina*. Minor clusters included a sequence related to Actinobacteria (37), unknown cyanobacteria, and microalgae chloroplastic 16S rDNA sequences. Few band sequences showed very low identity with those present in the database.

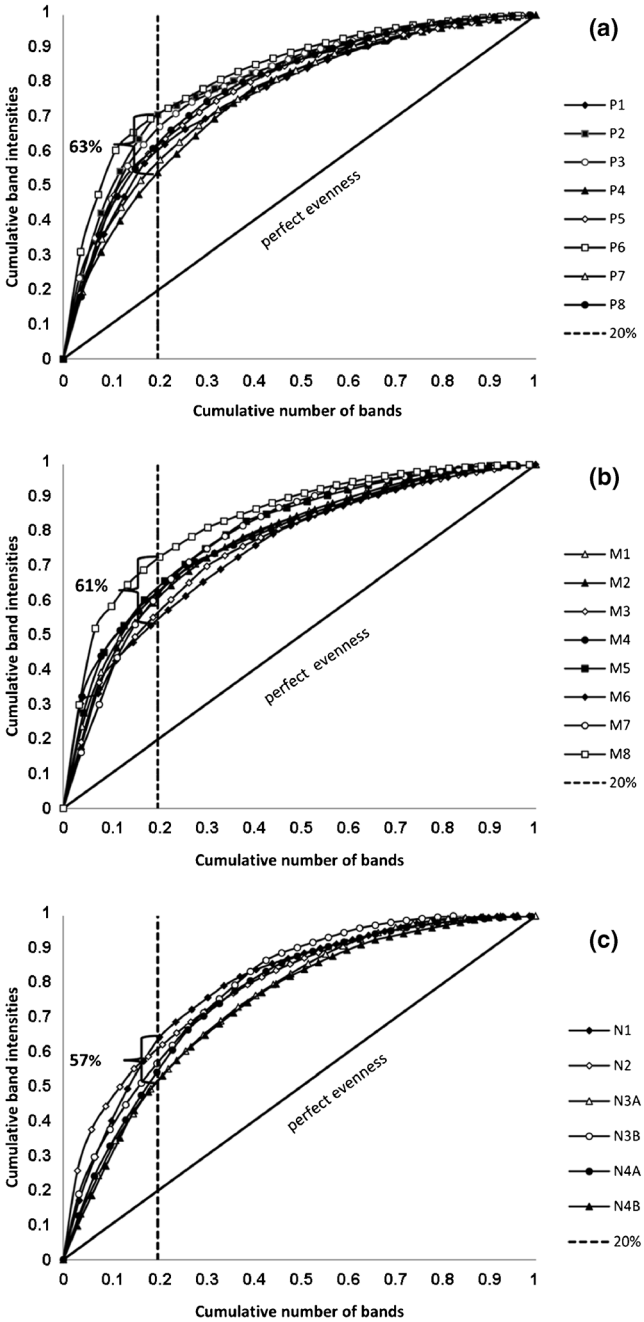

Fig. 4 Pareto-Lorenz distribution curves based on TGGE fingerprints of Kandalaksha Bay total bacteria communities in the various sampling sites. **a** *P* intertidal pool samples. **b** *M* water surface nearby the intertidal zone pool samples. **c** *N* open-sea samples taken at various depths. The *vertical lines* at the 0.2 x-axis (20 %) level are plotted to determine the functional organization indexes (*Fo*). Average *Fo* values are indicated as a percentage

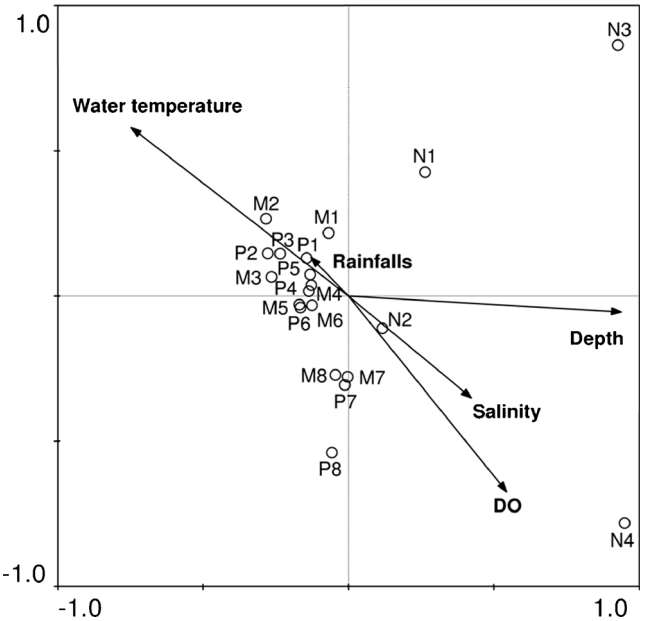

Fig. 5 Redundancy analysis (RDA) ordination diagram (biplot) showing samples (*numbered circles*) and significant ($p<0.05$) environmental parameters (*straight arrows*)

Discussion

The majority of microorganisms in nature (ca. 99 %) seem to be uncultivable. Possible reasons could be the lack of knowledge of their real growth conditions, phenomena of dormancy, inanition, or symbiosis (Fisher 1990; Amann et al. 1995; Muyzer 1999; Lewis 2007; Munn 2011). Therefore, for a better understanding of microbial diversity and its ecological role, it is advisable to use methods that are able to reveal the whole community, including uncultivable microorganisms (Muyzer and Smalla 1998; Muyzer 1999; Nicolaisen and Ramsing 2002). In this context, the PCR-TGGE experiments carried out in this study contribute to filling a large gap concerning the characterization of KB bacterial communities. In a previous work, we established that some physiological aspects of bacterial strains were strongly influenced by peculiar KB environmental conditions. These organisms developed uncommon adaptation responses that, generally, are not recognized in strains of the same species sampled elsewhere (Pesciaroli et al. 2012).

The bacterial community structure and its functional organization appeared to be in relation with KB hydrodynamics. Sea-surface communities were well homogenized, in particular on the coastal area, as indicated by both Pearson and Dice coefficients showing very little variations among P and M samples (Figs. 2b and 3b). Homogenization was probably due to strong water mixing that in KB is mainly caused by tide hydrodynamics rather than to wave motion (Pantyulin 2003). Apparently, tide mixing was so intense and effective that strong variations of meteorological conditions, such as rainfall, scarcely affected the communities' structure. This confirmed previous hydrodynamic data affirming that the KB main internal mixing factor is represented by tides, whose energy is distributed over the entire volume of the sea (Pantyulin 1990, 2003; Howland et al. 1999). Actually, the TGGE profiles of samples collected after heavy rains (high

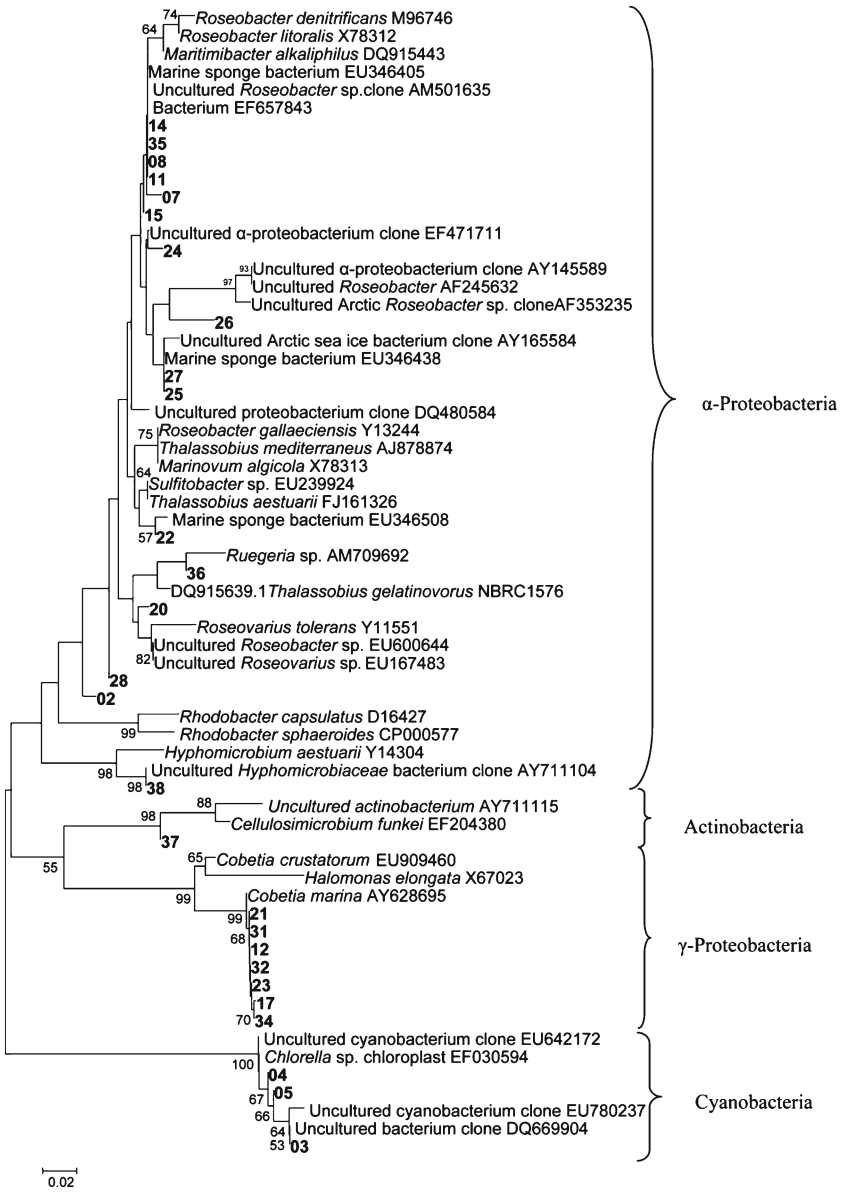

Fig. 6 Phylogenetic tree of Kandalaksha Bay bacterial community. The tree was inferred using the neighbor-joining algorithm based on 64 sequences and ca. 200 positions. Bootstrap values from 1,000 re-sampled data sets are shown: values below 50 % are not shown

input of freshwater and soil material) were very similar to those of samples withdrawn after a few days of dry weather (Table 1 and Fig. 2b). The scarce influence of rainfall was confirmed by the Canoco analysis (Fig. 5). By contrast, samples collected below the surface indicated that the mixing efficacy decreased proportionally to a depth producing an effect of vertical zonation. In fact, their TGGE profiles showed very low similarity with those from the coastal zone. Sample N2 was taken offshore from the sea surface (ca.10 km far from the P and M sampling site) and its band profile was more similar to the surface coastal samples than to those taken at different depths (Figs. 2b and 3b). These findings confirmed that water mixing and consequent bacterial community homogenization were intense at the surface on the whole bay, and that mixing effects were more evident closer to the shore. The limited mixing effects of tide on the deepest layers of the water column, already described by Savvichev et al. (2003), were confirmed by the great differences recorded for sample N4 (taken at −70 m). Also, multivariate analysis (CANOCO) clearly indicated depth as the main environmental parameter affecting the structure of KB bacterial communities. It is worth noting that sample distribution and segregation by the Canoco plot (Fig. 4) somehow reflected the clustering obtained by Pearson and Dice coefficients (Figs. 2b and 3b): samples N1, N3, and N4 were located far from all the others.

According to Marzorati et al. (2008), community diversity is described by the range-weighted richness (*Rr*) that depends both on the number of bands and on their distribution along the profile. Thresholds of $Rr<10$ and $Rr>30$ define communities with low and high diversity, respectively. In our case, even with differences related to the sampling location, KB showed quite high levels of bacterial diversity being always

Rr>30 (Table 2). Very habitable environments can host a lot of different organisms and genetic variability and, consequently, a wide TGGE gradient is necessary to accommodate its wide diversity (Marzorati et al. 2008). In our case, we obtained both high band numbers and complete profile coverage using a rather wide gradient; this indicated good environmental habitability. In other words, the KB environment showed a remarkable carrying capacity, being able to support a large number of different species, as confirmed also by the high values of samples diversity revealed by *Sd* that was always >0.8.

The community functional organization (*Fo*) indicated the presence of some dominant species with high abundance and many others showing lower abundance (Fig. 4). The various *Fo* reflected the community ability to be organized in adequate distribution of dominant and resilient microorganisms. Resilient strains are able to respond to perturbations by resisting damages and recovering quickly. These microorganisms can replace former dominant species in response to environmental changes. Thus, although slightly specialized (45 % < *Fo* < 80 %), KB communities showed good functionality and flexibility and the ability to rapidly react to changing and stressing conditions (Marzorati et al. 2008). These features can be considered consistent with the KB environment characterized by frequent variations of temperature, fresh water, and nutrient input, and strong water mixing (Howland et al. 1999; Berger and Gorbushin 2001; Savvichev et al. 2003; Dolotov et al. 2005). KB bacteria are generally well-adapted to very wide temperature variations (Pesciaroli et al. 2012); therefore, the community seems particularly apt to cope with the strong climate changes recently recorded in Arctic and sub-Arctic regions (Overland et al. 2011).

The phylogenetic analysis, based on sequences obtained by re-amplifying DNA from excised TGGE bands, showed the typical taxonomic configuration found in seawater with α- and γ-proteobacteria as major groups (Pommier et al. 2007). Most of the KB α-proteobacteria were located close to species of the genus *Roseobacter*. The great majority of known *Roseobacter* spp. is of marine origin, being well-represented across diverse habitats, from coastal to open oceans and from sea ice to hydrothermal vents (Buchan et al. 2005; Wagner-Döbler and Biebl 2006; Brinkhoff et al. 2008). Others have been isolated from saline and hypersaline environments (Labrenz et al. 2005; Martínez-Checa et al. 2005).

All KB γ-proteobacteria (the majority of them were retrieved from N samples) were related to *Cobetia marina*, known as a psychrotolerant species (Arahal et al. 2002; Yumoto et al. 2004). These microorganisms belong to the family *Halomonadaceae*, which consists mostly of marine halophilic or moderately halophilic bacteria found in temperate and Antarctic saline lakes and other marine environments (Kaye et al. 2004; Arahal and Ventosa 2006), and is associated with marine organisms (Ivanova et al. 2005). In our previous study, related to cultivable KB bacteria, predominant species belonged to *Pseudomonas* and *Serratia*, with a lower presence of *Sfingobacterium*, *Flavobacterium*, and other genera (Pesciaroli et al. 2012). It is worth noting that none of the TGGE excised bands could be related to these groups. This could be explained by the use of unspecific primers for PCR-TGGE. In addition, even if present in lower number within a composite community, a microorganism belonging to the mentioned species could overwhelm other more abundant bacteria due to their better growth under the conditions used for isolation.

In conclusion, although not exhaustive, this work represents the first attempt to characterize in detail the bacterial community present in the seawater of Kandalaksha Bay. PCR-TGGE fingerprinting and subsequent statistical analyses permitted us to obtain valuable information on its community structure and biodiversity in relation to various environmental parameters and tidal hydrodynamics. The results reported here can be used a solid base to carry out further investigations on specific issues for better biodiversity characterization of this sub-extreme environment.

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