ORIGINAL ARTICLE

Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean

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Microbial biogeographic patterns in the deep sea depend on the ability of microorganisms to disperse. One possible limitation to microbial dispersal may be the Walvis Ridge that separates the Antarctic Lower Circumpolar Deep Water from the North Atlantic Deep Water. We examined bacterial communities in three basins of the eastern South Atlantic Ocean to determine diversity and biogeography of bacterial communities in deep-sea surface sediments. The analysis of 16S ribosomal RNA (rRNA) gene clone libraries in each basin revealed a high diversity, representing 521 phylotypes with 98% identity in 1051 sequences. Phylotypes affiliated with *Gammaproteobacteria*, *Deltaproteobacteria* and *Acidobacteria* were present in all three basins. The distribution of these shared phylotypes seemed to be influenced neither by the Walvis Ridge nor by different deep water masses, suggesting a high dispersal capability, as also indicated by low distance–decay relationships. However, the total bacterial diversity showed significant differences between the basins, based on 16S rRNA gene sequences as well as on terminal restriction fragment length polymorphism fingerprints. Noticeably, both geographic distance and environmental heterogeneity influenced bacterial diversity at intermediate (10–3000 km) and large scales (> 3000 km), indicating a complex interplay of local contemporary environmental effects and dispersal limitation.

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Introduction

Biogeographic patterns in microbial communities are traditionally explained by two factors, the environmental heterogeneity and historical events (Martiny *et al.*, 2006; O'Malley, 2008). On the basis of the cosmopolitan hypothesis, 'everything is everywhere, but the environment selects' (Baas-Becking, 1934), environmental conditions have long been considered to have a strong influence on microbial biogeography. The effects of spatial distances (historical events) have been shown to affect microbial diversity in several studies (Papke *et al.*, 2003; Whitaker *et al.*, 2003; Martiny *et al.*, 2006; Ramette and Tiedje, 2007). The relative influences of environmental heterogeneity and historical events on microbial biogeography are still poorly understood. In marine habitats like the deep sea, microorganisms in the surface sediment may be assumed to disperse with oceanic currents.

Bioirrigation by the activities of larger benthic organism as well as near-bed currents (Hughes and Gage, 2004; Queric and Soltwedel, 2007) influence the sediment-water interface exchange and consequently lead to the dispersal of particles and therefore of microorganism. Barriers to microbial dispersal could be physical (topography) or physiological conditions (temperature, pH or hydrostatic pressure).

In the eastern South Atlantic Ocean, the Cape Basin is separated from the Angola and Guinea basins by the Walvis Ridge that forms a barrier to the northward and southward flow of water below a depth of about 3000 m (Shannon and Chapman, 1991). Furthermore, the Cape Basin is dominated by Lower Circumpolar Deep Water arriving from Antarctica and the deepest part of the Angola and Guinea Basins are filled with North Atlantic Deep Water originating from the Arctic (Bickert and Wefer, 1996). Noticeably, the Walvis Ridge has been shown to function as a barrier for the dispersal of some crustacean species of *Peracarida* (Brandt *et al.*, 2005), but it is not known whether this physical barrier also affects microbial dispersal.

To analyze whether different deep water masses associated with the physical barrier of the

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Walvis Ridge have significant structuring effects on microbial diversity, the bacterial diversity in three deep-sea basin surface sediments was determined by 16S ribosomal RNA (rRNA) gene sequencing and the community fingerprinting method terminal restriction fragment length polymorphism (T-RFLP). The relative contribution of environmental heterogeneity and of historical events on microbial biogeography were assessed for this data set in concert with earlier published data on basaltic lavas in the Pacific Ocean (Santelli *et al.*, 2008), shallow permanently cold sediment of the Arctic Ocean (Ravenschlag *et al.*, 1999) and Antarctic continental shelf sediment (Bowman and McCuaig, 2003).

Materials and methods

16S ribosomal RNA gene clone libraries construction

Sediment sampling was performed on the DIVA II cruise by a multicorer (Barnett *et al.*, 1984) in water depths ranging from 5032 to 5649 m. The sediment

cores were sliced on board in layers of 2 cm and the layers were subsampled top-to-bottom by sterile 1- to 2-ml syringes at 4 °C. After storage at -80 °C, DNA was extracted from 0.5 g of the surface sediment sample (0–2 cm) of the Cape, Angola and Guinea I areas (Figure 1, Table 2) after the protocol of the FastDNA SPIN Kit for Soil (Q-BIOgene, Carlsbad, CA, USA). Bacterial 16S rRNA genes were amplified using the primer pair GM3/GM4 (Muyzer *et al.*, 1995). The 100- μ l reaction contained 30 ng DNA as template, 0.5 μ M of each primer, 10 mM of dNTPs, 1 $\times$ buffer (Eppendorf, Hamburg, Germany) and 5 U of the Takara-Taq DNA polymerase (TAKARA, Dalian, China). PCRs were performed in 10 replicates with 20 cycles to minimize PCR bias. Final extension was performed 60 min at 60 °C to increase 3'-A-overhang. The amplicons were pooled and purified with a PCR purification kit (Qiagen, Hilden, Germany). Cloning of the amplicons was performed using TOPO TA Cloning Kit for sequencing (pCR4-TOPO, Invitrogen, Karlsruhe, Germany). Clones with a correct insert size of $\sim$ 1500 bp were

Figure 1 Sampling areas in the South Atlantic Ocean as well as the Walvis Ridge that separates the Cape Basin from the Angola and Guinea basins. For the 16S ribosomal RNA (rRNA) gene approach surface sediment (0–2 cm) of the Cape, Angola and Guinea I areas were used and for the terminal restriction fragment length polymorphism (T-RFLP) analysis 3–5 surface sediments of the Cape, Angola and Guinea I–III areas were analyzed.

sequenced using the vector primers M13 F (5'-GGAA ACAGCTATGACCATG-3') and M13 R (5'-GTTGTAA AACGACGCCAGT-3').

Phylogenetic and sequence analyses

The quality of the obtained sequences was manually checked using Sequence Analysis 5.2 (Applied Biosystems, Weiterstadt, Germany). Full-length sequences were assembled with Sequencher (Gene Code, Ann Arbor, MI, USA). No chimeras were detected with Bellerophon (Huber *et al.*, 2004) and CHECK_CHIMERA (Maidak *et al.*, 1996). Sequences were imported into the ARB software package (Ludwig *et al.*, 2004) and aligned using the ARB FastAligner, then refined manually. The ARB software package was used to generate phylogenetic trees of 810 full-length sequences using the maximum likelihood algorithm with a 50% positional conservation filter and with 100 bootstrap replicates. Sequences reported in this study were deposited at EMBL under the accession numbers AM997284–AM997988 for 705 full-length sequences and under AM997989–998333 and AM997283 for 346 partial sequences.

The software distance-based OTU and richness (DOTUR) was applied to ARB distance matrices generated with the Jukes-Cantor correction to estimate operational taxonomic units (OTU), rarefaction curves of observed OTUs, richness estimators and diversity indices (Schloss and Handelsman, 2005). A sequence identity of 98% was used to define OTUs, as this cut-off roughly corresponds to the species level (Rossello-Mora and Amann, 2001; Stackebrandt and Ebers, 2006). The statistical tool *j*-LIBSHUFF was applied to genetic distance matrices to determine whether differences in library composition were because of chance or to biological effects, and significances were assessed by Monte Carlo permutations and further corrected for multiple comparisons (Schloss *et al.*, 2004). The statistical tool SONS (Schloss and Handelsman, 2006) was used on full-length 16S rRNA gene sequences to calculate Chao1 shared richness estimates, the J_{class} index for the ratio of shared to total number of OTUs, and θ_{yc} for the estimated similarity in community structure between any two communities.

Terminal restriction fragment length polymorphism

Terminal restriction fragment length polymorphism analyses included three to five samples of surface sediments (0–2 cm) from several cores of each area, Cape, Angola and Guinea I–III (Figure 1, Table 2). Genomic DNA was extracted from 0.5 g sediment samples using the FastDNA Spin Kit for Soil (Q-Biogene, Irvine, CA, USA). PCR amplification of the 16S rRNA gene was carried out using the fluorescently labelled primers 27F (FAM, 5'-AGAGTTTGA TCCTGGCTCAG-3') and 907R (HEX, 5'-CCGTCAAT TCCTTTTRAGTTT-3'), targeting all bacteria as well

as 558F (FAM, 5'-ATTGGGTTTAAAGGGTCCG-3') (Abell and Bowman, 2005a,b) and 1390R (HEX, 5'-GACGGGCGGTGTGTACAA-3') (Zheng *et al.*, 1996), targeting the class *Flavobacteria*. Undigested and digested amplicons were identified by capillary electrophoresis to verify the absence of false-positive fragments in the undigested control and the completeness of the digestion. PCRs were carried out in a total volume of 25 μ l, including 12.5 μ l PCR Master Mix (Promega GmbH, Mannheim, Germany), 1 μ M forward and reverse primer, and 5–24 ng DNA template. PCR reactions were carried out in triplicates and purified on Sephadex columns (Sephadex G-50 Superfine, Amersham Biosciences AB, Uppsala, Sweden). PCR amplicons (70–120 ng) were digested in a total volume of 10 μ l at 37 °C for 3 h using 5 U of the restriction enzyme *AluI* (Fermentas, Burlington, Canada) for bacterial amplicons and 5 U of the enzyme *MspI* (Fermentas) for *Flavobacteria* amplicons. The two restriction enzymes were chosen based on high numbers of unique terminal restriction fragments assessed with *in silico* analyses using enzyme restriction power analysis (<http://mica.ibest.uidaho.edu/>) as well as on best performance in laboratory experiments (that is, producing maximum numbers of terminal restriction fragments (TRFs)). After heat inactivation (65 °C, 25 min) and purification on Sephadex columns, detection of TRFs was performed on a ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) equipped with a 80-cm capillary, a POP-7 polymer and the filter set DS-30. The ROX-labelled MapMarker 1000 (Eurogentec, Seraing, Belgium) served as a size standard between 50 and 1000 bp. The fragment profiles were visualized and automatically analyzed with GeneMapper v. 3.7 Software (Applied Biosystems), using standardized settings with a peak detection cut-off set to 30 fluorescence units. The 5'-end labelled TRFs were used as they produced a higher number of fragments in comparison with 3'-end TRFs (Suzuki *et al.*, 1998; Osborn *et al.*, 2000).

A binning procedure was applied to the GeneMapper output to compensate for slight peak shifts between runs and for TRF size calling imprecision, in order to avoid artificial, technically derived differences between profiles (Hewson and Fuhrman, 2006). The technical variability of peak size calling in different replicates including runs conducted on different days was determined as of ± 0.25 bp (window size of 0.5 bp). The binning function included two different starting points (50 and 50.25 bp) and the binning strategy yielding higher correlation between all samples was selected for further statistical analyses. The binning window was adjusted to 1 bp for samples amplified with *Flavobacteria* primers, because a window frame of 0.5 bp did not yield higher resolution. The computation was carried out with the Interactive Binner function (Ramette, 2009 <http://www.ecology-research.com>). The output consisted of a table of TRFs with

corresponding relative fluorescence intensities, which are the individual peak area divided by the total area of peaks in a given profile. Master profiles were generated by building a consensus table of the binned TRF profiles for all samples from one basin, averaging the respective relative fluorescence intensities values of all samples. A TRF was considered present if it appeared in one or more PCR parallels, therefore including all natural and technical variability at this level of analysis.

Statistical analyses

Non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) were carried out with the program PAST (Paleontological Statistics, ver. 1.47, <http://folk.uio.no/ohammer/past>). Simple and partial Mantel tests were used to determine the significance and correlation coefficients between genetic-, spatial- and environmental distance matrices, using the R package *vegan* (<http://vegan.r-forge.r-project.org/>) (Legendre and Legendre, 1998; Mantel, 1967). Spatial dissimilarities based on geographic distances between sites and environmental dissimilarities (temperature, salinity, pH, Eh, TOC, Chl *a* and grain size; Table 1) were used to explain genetic dissimilarity. To determine the strength of the relationship between genetic and geographic distance linear models were fitted and slope coefficients were calculated with their 95% confidence intervals.

Results and discussion

Bacterial biomass and richness in sediments of the South Atlantic Ocean

The cell numbers of the suboxic surface sediments (0–2 cm) in three eastern South Atlantic Ocean basins were $3.4\text{--}3.7 \times 10^9$ cells g⁻¹ sediment (Table 1). The abundances were in the range found in other deep-sea sediments (9.2×10^8 cells g⁻¹ (Deming and Colwell, 1982), 1.5×10^9 cells g⁻¹ (Guezennec and Fiala-Medioni, 1996) and 5×10^8 cells g⁻¹ (Harvey *et al.*, 1984)). The 16S rRNA gene libraries showed a high diversity with up to 20 different phyla in the Cape Basin and 17 phyla in the Angola and Guinea basins (Figure 1). Earlier described

deep-sea (Bowman and McCuaig, 2003; Polymenakou *et al.*, 2005, 2009; Xu *et al.*, 2005) and shallow sediments (Ravenschlag *et al.*, 1999) had also found a large diversity, which may be based on a weak and symmetric competition (Grant, 2000). The reciprocal Simpson's indices for all sites were above 50 (Table 2), suggesting evenly distributed diversity profiles as typical dominance profiles show 1/*D* values below approximately 50 (Zhou *et al.*, 2002). Total richness estimates (Chao1) (Table 2) and rarefaction curves (Supplementary Figure S1) based on a 98% sequence identity showed that Cape, Angola and Guinea basin surface sediments contained an equal bacterial richness at a significance level of 0.05.

Both analyses predicted a lower richness for the South Atlantic sediments in comparison to the Antarctic sediments and a higher richness in comparison to the Arctic sediment. The library-based equality of richness was supported by the T-RFLP analysis, as basin-specific master profiles showed a comparable OTU richness (167, 190 and 182 TRFs for the Cape, Angola and Guinea Basin, respectively) (Figure 4a).

Bacterial diversity of the 16S ribosomal RNA genes

The clone libraries contained 521 phylotypes with 98% identity in 1051 sequences, containing 705 full-length sequences. Applying a 100% identity threshold revealed 230 sequences, which were present at least twice, with a majority of 176 sequences (18 OTUs) present in all deep-sea sediments. The bacterial communities were dominated by *Proteobacteria*, which accounted for 64, 58 and 63% of all sequences in the Cape, Angola and Guinea Basin, respectively, with the class *Gammaproteobacteria* representing 45, 37 and 40% of all sequences in the respective basins (Figure 2). The class *Gammaproteobacteria* comprised 116 phylotypes (98% identity, 427 sequences), of which 39 phylotypes (138 sequences) were related to known cultivated species. These belonged mainly to families of psychrophilic microorganisms including *Enterobacteriaceae*, *Alteromonadaceae*, *Oceanospirillaceae* and *Legionellaceae* (Figure 3a). Among

Table 1 Sediment data (Türkey and Kröncke, in preparation) and cell numbers of microbial communities in the South Atlantic Ocean

Basin	Depth (mbsl) ^a	Temp. (°C)	Salinity (‰)	pH	Eh (mV)	TOC (%)	Chl <i>a</i> (µg g ⁻¹)	Grain size (%)		Cell counts (cell g ⁻¹)	MPN (cells ml ⁻¹)
								< 63 µm	> 63 µm		
Cape	5032	1.14	34.6	7.74	177	0.83	0.017	92.89	6.87	3.5×10^9	1.22×10^4
Angola	5649	ND	ND	7.72	96	0.9	0.069	83.84	16.4	3.4×10^9	2.67×10^5
Guinea I	5063	2.1	34.9	7.77	183	0.72	0.264	84.23	15.23	3.7×10^9	2.67×10^4
Guinea II	5225	ND	ND	7.76	132	0.77	0.301	84.99	14.46	ND	ND
Guinea III	5525	2.1	34.5	ND	ND	0.76	0.152	86.45	13.34	ND	ND

Abbreviation: ND, not detected.

^aMeters below sea level.

Table 2 Sampling sites of sediments used for 16S rRNA gene sequencing or for T-RFLP analysis with corresponding richness and diversity indices for bacteria

Sampling area	Latitude	Longitude	Depth (mbsl) ^a	Stations ^b T-RFLP	Stations ^b 16S rRNA gene seq.	No. of clones ^c	OTU DOTUR 0.02	Richness estimator Chao1 ^d	Simpson 1/D	Study
Cape	28°06'42" S	7° 20'48" E	5032	33, 34, 37, 38	33	342 FP 228 F	202 145	466 (369, 620) 508 (351, 785)	77 53	This study
Angola	9°56'00" S	0° 53'48" E	5649	46, 48, 50	46	354 FP 219 F	183 126	256 (227, 305) 369 (259, 570)	77 59	This study
Guinea I	0°00'00" S	2°25'06" W	5063	56, 58, 59, 60, 61	60	355 FP 258 F	203 155	369 (308, 465) 489 (348, 735)	125 91	This study
Guinea II	0°50'00" N	5° 35'00" W	5225	74, 75, 76, 77, 79	—	—	—	—	—	This study
Guinea III	0°37'12" N	6° 28'06" W	5525	95, 97, 99	—	—	—	—	—	This study
Antarctic continental shelf	66°31'86" S	143°38'30" E	761	—	MERTZ 0–2 cm	590 P	322	899 (713, 1175)	125	Bowman and McCuaig, 2003
Arctic ocean Svalbard	79°42'81" N	11°05'18" E	218	—	Station J	123 P	84	125 (104, 167)	167	Ravenschlag et al., 1999
East Pacific	9°28'48" N	104°13'48" W	2516	—	EPR	370 F	239	601 (475, 796)	200	Santelli et al., 2008
Rise Hawaii	–9°50'38" N 18°52'17" N	–104°17'86" W 155°14'53" W	–2674 888	—	PV	472 F	276	764 (597, 1017)	167	Santelli et al., 2008
	–18°58'31" N	–155°53'42" W	–1714							

Abbreviations: DOTUR, distance-based OTU and richness; OTU, operational taxonomic units; rRNA, ribosomal RNA; T-RFLP, terminal restriction fragment length polymorphism.

^aMeters below sea level.

^bFor details see cruise report DIVA II (M63/2).

^cNumber of full-length (F) and partial (P) sequences, full-length sequences and values calculated from them are presented in bold.

^dChao1 richness with lower and upper bound of 95% confidence interval.

these phylotypes 11 OTUs (12 sequences) clustered with the NOR5/OM60 clade that includes '*Congregibacter litoralis*' strain KT71, the first marine aerobic anoxygenic phototrophic *Gammaproteobacteria* in culture (Fuchs et al., 2007; Yan et al., 2009). Three phylotypes (5 sequences) were related to free living (*Thiothrix*) and endosymbiotic sulfur oxidizers and methylotrophic bacteria. A large portion of 77 phylotypes (289 sequences) clustered distinctly from cultured species to JTB255/BD3-6 (38 phylotypes, 192 sequences), BD7-8/MERTZ (10 OTUs, 36 sequences), JTB23/Sva0091 (18 OTUs, 34 sequences) (Figures 3a and b) and to Cret-1F, BD1-1, PWP and South Ionian groups (11 OTU, 27 sequences). These groups included only 16S rRNA gene sequences that originated from other deep-sea or permanent cold marine habitats (Kato et al., 1999; Li et al., 1999; Ravensschlag et al., 1999; Urakawa et al., 1999; Bowman and McCuaig, 2003; Polymenakou et al., 2005; Xu et al., 2005; Zhao and Zeng, 2005).

The *Alpha*-, *Beta*- and *Deltaproteobacteria* accounted together for 18 to 23% of all sequences in the libraries. *Deltaproteobacteria* (11 to 14%) outnumbered *Alphaproteobacteria* (6 to 8%) and *Betaproteobacteria* (1 to 3%) (Figure 2). Other groups with a sequence abundance of over 5%, which occurred in all three basins, were the

phyla *Chloroflexi* (1, 10 and 4% for Cape, Angola and Guinea basins, respectively), *Planctomycetes* (6, 4 and 10%), *Acidobacteria* (4, 7 and 5%) and *Bacteroidetes* (10, 4 and 6%).

Bacterial diversity comparison

The proportion of bacteria present in two or three basins was high in the 16S rRNA gene sequences analyses (23%) and in the T-RFLP analyses (58%) (Figure 4a). A third of the fragments (93 TRFs) was detected in the sediments of all basins and represented 82% of the total relative fluorescence intensities. Among the 16S rRNA gene sequences, a shared membership of 19 OTUs (98% identity) was found in all three basins with the statistical tool SONS. The manual assignment in ARB confirmed the small fraction of OTUs detected in all three basins (29 OTUs, 347 sequences), but provided additional information regarding the sequence abundance and identity of each OTU. These were dominated by *Gammaproteobacteria* (76%; Figure 4b). In this class, the common members were related to marine heterotrophic aerobic and facultative anaerobic microorganisms (*Alteromonadaceae* and *Oceanospirillaceae*), photoheterotrophic aerobic bacteria (NOR5/OM60 clade) (Fuchs et al., 2007), and to groups consisting of uncultivated bacteria

Figure 2 Bacterial diversity in the Cape Basin (342 sequences), Angola Basin (354 sequences) and Guinea Basin (355 sequences). All detected classes in the domain *Bacteria* are shown.

(JTB255/BD3-6, JTB23/Sva0091/BD3-1, BD7-8/MERTZ, Gret-1F and South Ionian).

Phylotypes present in two of three basins belonged to the *Gamma*- and *Deltaproteobacteria* and to the *Chloroflexi*. A major group of *Chloroflexi*-OTUs were restricted to Angola and Guinea basin sediments (7 OTUs, 21 sequences). The *f*-LIBSHUFF analyses revealed no significant difference between the Angola and Guinea libraries as well as Cape and Guinea libraries (using a minimum *P*-value of 0.0012) (Supplementary Table S1). Thus, common phylotypes dominate the communities of these basins. The largest number of TRFs covered by two basins was found for the Angola and Guinea basins (30 TRFs, 30.5 relative fluorescence intensity) (Figure 4a). High chlorophyll *a* contents were detected in the Angola and Guinea surface sediments indicating a large fraction of fresh, recently arrived organic carbon (Table 1, Türkay and Kröncke, in preparation). This probably originated from a primary productivity in the surface waters that can be linked to the discharge of nutrients from the Congo and the Niger Rivers into the Angola and Guinea basins, respectively (Schefuss *et al.*, 2004).

Angola and Cape basins showed significantly different communities (*f*-LIBSHUFF test, *P* = 0.008)

and significantly different *Flavobacteria* T-RFLP profiles (Figure 5b) (analysis of similarity, *R* values 0.869, *P* < 0.001) (Supplementary Table S2). These differences were consistent with a different chlorophyll *a* content as well as a different sediment particle size in the Cape Basin (Table 1) (Etter and Grassle, 1992), indicating that environmental factors seem to influence bacterial communities in deep-sea sediments of the eastern South Atlantic Ocean. It is, however, needed to also take spatial parameters into account in this analysis to strengthen our interpretation concerning environmental or spatial effects on the observed community shifts.

Biogeography: environmental and historical factors

In the eastern South Atlantic Ocean the Walvis Ridge separates the Cape Basin from the Angola and Guinea basins below a depth of about 3000 m and causes different deep water masses in these basins. The dominance of common phylotypes in the 16S rRNA gene libraries and T-RFLP master profiles suggested that microbial dispersal may not be influenced by the Walvis Ridge or by the presence of different water masses. This was

To get more information regarding the amount of spatial structure present, we analyzed the relative relationships between genetic diversity and geographic distances. The 16S rRNA gene and TRFs based distance-decay relationships for the South Atlantic Ocean and for all sites were all

From the clustering of TRF profiles by basins, as shown by non-metric multidimensional scaling (Figure 5a) associated with large, significant R values for all pairwise comparisons between the deep-sea basins (analysis of similarity, 0.586 to 0.999, $P < 0.001$) (Supplementary Table S2), and from significant differences between the South Atlantic Ocean communities to all other communities (β -LIBSHUFF tests, Supplementary Table S1), it seemed obvious that communities were structured either by the contemporary environment, spatial distances (historical events) or by a combination of both (Martiny *et al.*, 2006; Ramette and Tiedje 2007).

Figure 4 (a) Shared and basin-specific SONS generated OTU_{0.02} and terminal restriction fragment (TRF) and corresponding relative fluorescence intensities (RFI) values for the three deep-sea basin of the South Atlantic Ocean. (b) Phylogeny of the shared OTUs between three and two deep-sea basins and the corresponding number of sequences.

To disentangle the relative influence of environmental heterogeneity and spatial distance on the distribution of microbial deep-sea sediment communities, we used a combination of simple and partial Mantel tests. For distances of 0–1200 km T-RFLP results showed a comparable influence of both factors (environment $r=0.636$, $P<0.001$, geography $r=0.651$, $P<0.001$) (Table 2) (Figure 6b).

But environment ($r=0.588$, $P<0.001$) overwhelmed any effect of geographic factors ($r=0.278$, $P=0.009$) for intermediate distances (1200–3500 km), as also supported by significant partial Mantel tests (Table 3). A higher correlation between spatial and genetic distance for small spatial scales (<200 m) was reported for other microbial groups in soil (Cho and Tiedje, 2000), suggesting the existence of

Figure 5 (a) Non-metric multidimensional scaling (nMDS) plot using Bray–Curtis distance for the data set derived from amplification with bacterial primers 27F and 907R and subsequent digestion with *AluI*. Stress: 0.15. (b) nMDS plot using Bray–Curtis distance for the data set derived from amplification with *Flavobacteria*-specific primers 517F and 1457R and digestion with *MspI*. Stress: 0.07.

endemic taxa, as genetic distance increases with spatial distances.

Mantel tests for 16S rRNA gene sequences revealed that both environment and geography ($r = 0.008$, $P = 0.006$ and $r = 0.024$, $P = 0.001$, respectively) had an influence on the bacterial diversity of the South Atlantic. Significant correlations between genetic and geographic distances (Mantel's coefficient $r = 0.013$, $P = 0.001$) (Table 3, Figure 6a) were as well observed for all deep-sea and coastal sediments.

When compared in more detail, the two methods showed different results for the relative influence of both factors on microbial biogeography. These detected differences reflect different levels of similarity, saturation and resolution of each method and sampling effort, for example, the T-RFLP analyses including Guinea I versus Guinea I+II+III (Table 3). Indeed, the analysis of 16S rRNA gene sequences

Table 3 Slope coefficients and Mantel r statistics for genetic distance matrices derived from 16S rRNA gene sequences or T-RFLP

	Slope coefficient (genetic versus geographic distance)	Simple Mantel tests				Partial Mantel tests		Number of samples
		Geography ^a		Environment		Geography	Environment	
		<i>Log</i> ₁₀ transformed		Untransformed	<i>Log</i> ₁₀ transformed			
		Untransformed	<i>Log</i> ₁₀ transformed					
<i>South Atlantic, 0–1200 km distance</i>								
T-RFLP	28.28 [21.34, 35.22] ^b	0.074 [0.053, 0.095]	0.651 (<0.001)*	0.593 (<0.001)*	0.657 (<0.001)*	0.054 (0.306)	0.131 (0.134)	14
<i>South Atlantic, 0–3500 km distance</i>								
16S rRNA gene	0.19 [0.17, 0.22]	0.0036 [0.0028, 0.0044]	0.024 (0.001)*	0.012 (0.001)*	0.008 (0.006)*	0.031 (0.002)*	−0.021 (0.981)	3
T-RFLP (Guinea I)	7.5 [5.38, 9.62]	0.076 [0.063, 0.090]	0.698 (0.004)*	0.841 (0.001)*	0.886 (0.001)*	−0.138 (0.86)	0.768 (<0.001)*	11
T-RFLP (Guinea I+II+III)	2.6 [1.24, 3.96]	0.055 [0.042, 0.068]	0.278 (0.009)*	0.532 (0.001)*	0.599 (<0.001)*	−0.227 (0.992)	0.573 (<0.001)*	19
<i>All sites, 0–18 000 km distance</i>								
16S rRNA gene	0.02 [0.019, 0.022]	0.0030 [0.0027, 0.0032]	0.013 (0.001)*	0.013 (0.001)*	NA	NA	NA	7

Abbreviations: NA, no environmental parameters available for all comparisons; rRNA, ribosomal RNA; T-RFLP, terminal restriction fragment length polymorphism. For simple and partial Mantel tests, P -values based on 1000 permutations are indicated in parentheses with * when the P -values are still significant after Bonferroni correction for multiple comparisons.

^aFor simple Mantel tests, geographic distances were either left untransformed or $\log_{10}$ transformed to allow for comparisons with previously published studies.

^bLower and upper bounds of 95% confidence interval for the slope coefficients are indicated within square brackets.

Figure 6 Scatterplots with loess curve presenting (a) genetic dissimilarity plotted against geographic distance for genetic dissimilarity of distance matrices derived from 16S ribosomal RNA (rRNA) gene sequences of samples of the South Atlantic and (b) for terminal restriction fragment length polymorphism (T-RFLP) profiles of samples from five different stations in the South Atlantic (Cape, Angola, Guinea I, Guinea II and Guinea III).

provides information regarding randomly chosen phylotypes ('sampling communities') where the finding of an OTU is proportional to its abundance in the clone library (Bent and Forney, 2008). In contrast, the fingerprinting method T-RFLP screens for all OTUs present above the detection threshold of the method ('screening' communities; Bent and Forney 2008), typically $>6 \times 10^2$ – 10^3 DNA fragment copies per ml samples (Ramette 2009), but does not provide clear taxonomic distinction (Dunbar *et al.*, 2001).

Although high dispersal rates were detected for some groups in deep-sea sediments, both T-RFLP and 16S rRNA-based analyses suggest barriers for the dispersal of microorganisms in the deep sea.

The influence of both factors at intermediate scales was already shown by other studies (Green *et al.*, 2004; Reche *et al.*, 2005; Yannarell and Triplett, 2005), but our study suggest an effect of both factors for large scales as well, as shown for soil microbial communities (Fierer and Jackson, 2006). Although the small size, high dispersal rates, large population size and low extinction rates of microorganisms suggest a low effect of geographic barriers on microorganisms (Staley and Gosink, 1999; Beja *et al.*, 2002; Finlay, 2002; Ramette and Tiedje, 2007), our study shows that the distribution of microorganisms in deep-sea sediments is limited at intermediate (10–3000 km) and large scales (>3000 km).

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