Phylogenetic Variation in Bacterial Populations from Ten Atoll Lagoons in the Tuamotu Archipelago, French Polynesia.

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ABSTRACT

Atoll lagoons present unique opportunities to investigate factors controlling ecological interactions and microbial community composition. They are analogous to estuaries in that they are shallow water environments subject to restricted exchange with the surrounding ocean. Unlike estuaries, they are contained by barriers of uniform composition (calcium carbonate), the surrounding ocean is uniform (unlike most coastal/continental shelf waters) and terrestrial communities in the surrounding (small) watershed are fairly simple and uniform. We examined physiographic and environmental factors as well as the composition of microbial communities in ten lagoons of the Tuamotu archipelago, French Polynesia, by PCR/DGGE. Microbial populations of some lagoons were very similar, despite differences in activity, whereas others had distinct populations with dominance by a few bands. Seasonality and water exchange (and variables affected by exchange) appear to be the major factors controlling community composition in these lagoons.

Introduction

Coral reef atolls are unique habitats created by the interaction of biological processes and the physical environment. The result is a shallow water ecosystem (the atoll lagoon) surrounded on all sides by a uniform deep-water pelagic marine ecosystem. Although atolls differ in size and shape, the watershed they represent is generally small relative to the area of the lagoon they enclose. In addition, the composition of the terrestrial component of these ecosystems is relatively uniform. From an experimental standpoint, they present an opportunity to study ecological and biogeochemical processes in shallow water communities without the confounding influence of varying inputs from the surrounding watershed that complicate studies of continental estuarine ecosystems.

The Tuamotu-Gambier archipelago is a group of 77 atolls in French Polynesia. The islands are located in the south Pacific Ocean between 15^o and 25^o S latitude and 135^o and 150^o W longitude. Recently, the lagoons of some of these atolls have been the focus of studies conducted by the French agency ORSTOM to advance understanding of the factors controlling fish and shellfish production in the lagoons (the TYPATOLL program). In addition to measuring primary production and nutrient concentrations [1, 2], components of these studies have examined dissolved organic carbon distributions [10] and microbial loop processes [5, 12, 13, 14] in some of these lagoons.

We were interested in comparing the composition of the bacterioplankton in these lagoons to assess variability and to relate distributions to environmental factors. Our initial hypothesis was that the composition of the bacterioplankton would be similar between

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lagoons, since the physical environments (sediment composition, terrigenous inputs, climate, ocean water quality) were similar between lagoons. While this was true in general, we found that water residence time in the lagoons (and biological and physical properties deriving from it) appear to be important determinants of bacterioplankton composition. This results not from simple dilution with uniform oceanic populations (which were quite different from lagoon populations in all but one case), but rather from divergence of the biological and chemical characteristics of the lagoon ecosystem at long residence times.

Materials and Methods

Samples were collected in ten atoll lagoons during TYPATOLL Cruise 3 (November 1995, end of the dry season) and 4 (March and April 1996, end of the rainy season). Additional samples were collected during a visit to Tikehau lagoon in October of 1995. The environmental data discussed here were collected by others and are described more fully in other publications [1, 4, 10, 12, 13].

The sample collection and processing protocol for determining bacterioplankton composition was the same as that described in [3]. Briefly, samples were collected by filtering (200-300 kPa pressure) 2-5 liters of surface water through 0.45 μm pore size Sterivex® (Millipore) filter cartridges. Excess water was dispelled and the filter cartridges were filled with extraction buffer, sealed and frozen (initially at –20 ^{o}C, later at –80 ^{o}C). DNA was extracted from the samples and used in mixed-template polymerase chain reaction (PCR) amplifications with 17mer primers specific for the variable 3 region of the 16S rRNA gene: positions 341-358 (primer 341f, Bacteria); and 517-534 (primer 534r, Universal) in the *E. coli* gene. This primer set was chosen to amplify most species of bacteria [6, 8]. Primer 341f also contained a 40bp GC-clamp [9]. The products of the amplification were then resolved by denaturing gradient gel electrophoresis (DGGE) [7, 8, 9].

Gel images were recorded digitally, then processed using software marketed by Bio-Rad Inc. (Molecular Analyst Fingerprint Plus®, which appears to us to be the same as GelCompare®, marketed in Europe by Applied Maths; Kortrijk, Belgium). Samples (lanes) were compared using the regression-based correlation matrix routine in the cluster analysis module of the program. This similarity analysis uses the set of pairs of intensity values (y_{xa}, y_{xb}) for each position (x) on densitometric curves a and b to calculate a Pearson product-moment correlation coefficient for that pair of curves. The matrix of correlation coefficients for all pairs of curves is then used to generate a cluster analysis dendrogram (UPGMA).

Results and Discussion

An image of one of the DGGE gels used to analyze microbial community composition is shown in Fig. 1a. The samples in this gel are from some of the lagoons sampled in March and April on TypAtoll Cruise 4. Comparison of the banding patterns in the "STANDARD" lanes provides a visual indication of the repeatability of the analysis.

The samples grouped into 3 major clusters with similarities greater than 70% (Fig. 1b; a similar analysis of 5 replicate samples from California coastal waters clustered at 94%). One group (Cluster 1) includes all of the samples from Tikehau lagoon collected in October and samples from Kauehi and Nihiru lagoons collected in November during

TypAtoll 3. The second cluster (Cluster 2) includes ocean samples collected on both cruises and the sample from Tekokota lagoon collected on TypAtoll 3. The third cluster

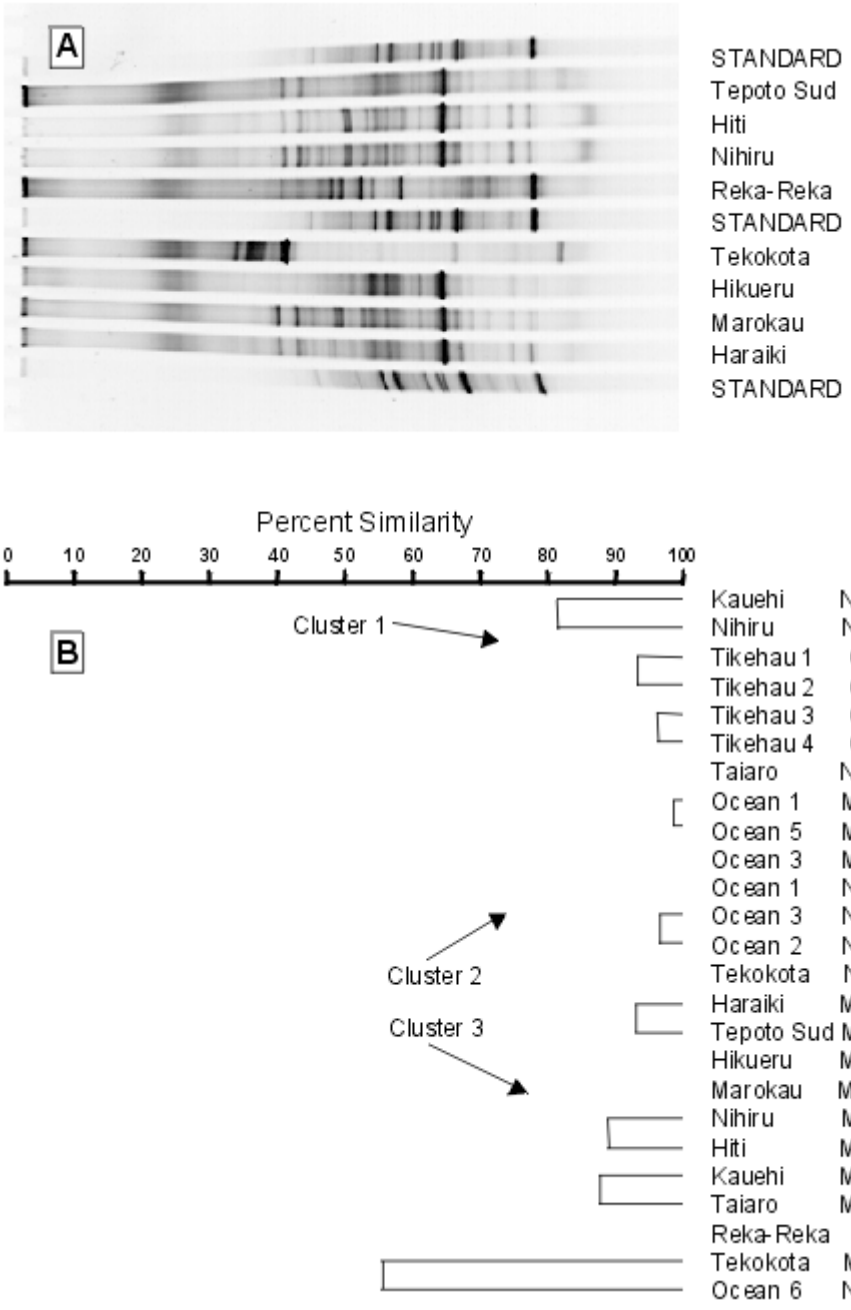

Fig. 1. a) Unprocessed image of a DGGE gel containing some of the samples collected on the TypAtoll 4 cruise in March 1996. b) Dendrogram showing similarity of samples collected in this study.

(Cluster 3) is composed of samples from Haraiki, Tepoto Sud, Hikueru, Marokau, Nihiru, Hiti lagoons collected during TypAtoll 3. The Kauehi and Taiaro lagoon samples collected on this cruise branched from Cluster 3 at 66% similarity. Similarities of the banding patterns for those samples included in the gel shown in Fig. 1a reflect this clustering.

Samples taken at different locations in Tikehau lagoon clustered at 84% similarity, indicating spatial homogeneity of the microbial assemblage in this lagoon. Tikehau lagoon samples (collected in October) clustered at 73% similarity with samples from Kauehi and Nihiru lagoons collected in November on TypAtoll cruise 3. We did not revisit Tikehau lagoon during the TypAtoll 4 cruise, but samples from Kauehi and Nihiru collected on this cruise clustered separately from the November samples (clusters linked at 52% similarity). This suggests a uniform seasonal shift in the composition of the microbial assemblages in these lagoons.

Some of the samples did not cluster closely with any of the others. The sample from Taiaro lagoon collected on TypAtoll 3 was most similar (branching at 61% similarity) to samples from Cluster 1. The sample collected from Reka-Reka lagoon on the TypAtoll 4 cruise was very different from other samples (branching from Clusters 1-3 at 12% similarity). The banding pattern for this sample (Fig. 1a) is clearly different from the rest of the samples collected on this cruise. Samples from Tekokota lagoon and Ocean Station 6 collected on TypAtoll 4 were the least similar to the rest of the samples. The low richness (number of bands, Fig. 1a) in the Tekokota lagoon sample is surprising because the sample collected from Tekokota lagoon during TypAtoll 3 (not shown) contained more bands, did not have any strongly dominant bands and closely resembled (92% similarity) the ocean samples collected on this cruise (Fig. 1b). The pattern shown here is similar to patterns seen with pure cultures and may indicate either that this sample was taken from a bloom (c.f. [11]) or that it was contaminated. The banding pattern for the sample collected at Ocean Station 6 in November (not shown) was also dominated by an intense band at the same position in the gel as the Tekokota lagoon sample; however, it contained additional bands at positions corresponding to bands in the other ocean samples.

As discussed by Charpy et al.[1] and Pages et al. [10], the environmental characteristics of these lagoons (chlorophyll concentration, color, dissolved and particulate organic matter quantity and quality) represent a gradient with oceanic conditions as one end-member. Water quality in Tekokota lagoon was the most oceanic and Taiaro lagoon the least oceanic of the lagoons we sampled (Table 1). Microbial community composition also follows this pattern. The sample collected at Tekokota lagoon during TypAtoll 3 (we are discounting the sample collected during TypAtoll 4 for reasons given above) clustered very closely with oceanic samples collected on that cruise. This is a small lagoon with high porosity and water quality very similar to oceanic, suggesting a short water residence time. Microbial populations might thus be expected to be similar to those in the surrounding ocean as they have little time to change.

In contrast, water quality of both Taiaro and Reka-Reka lagoons deviates substantially from oceanic (Table 1). These are also small lagoons, but with low porosities suggesting long water residence times. Although water quality variables indicate that Taiaro lagoon is the least oceanic, the Reka-Reka lagoon microbial assemblage had the lowest similarity to assemblages in the other lagoons or to ocean assemblages. In addition, the thymidine incorporation rate measured in Reka-Reka lagoon was markedly greater than rates measured in other lagoons, including Taiaro lagoon. Although Taiaro lagoon had higher dissolved organic carbon concentrations, chlorophyll concentrations were higher in Reka-Reka lagoon. Data presented Torreton et al [14] indicate that Reka-Reka lagoon populations were strongly P-limited. Microbial assemblages from Taiaro lagoon were

more similar to those of the other lagoons sampled at the same time and grouped loosely with them.

Table 1. Characteristics of Tuamoto Archipelago atolls sampled during TypAtoll cruises 3 and 4. Data were either collected during this study or are taken from papers by [1]Charpy et al. (1997); [2]Pages et al. (1997); or [3]Torreton et al. (In press), Table 4.

ATOLL	Area[1] (km^2)	Lagoon Area[1] (km^2)	Lagoon depth[1] (m)	Number of Inhabitants[1]	Porosity[1]	Chlorophyll a <3µm[1] (%)	Chlorophyll a[1] ($\mu g\ l^{-1}$)	A_{254}[2] (m^{-1})	Oceanic Optical Index[2]	DOC[2] (µM)	DON (µM)	DOP (µM)	POC[1] (µM)	PON[1] (µM)	TdR (pM hr^{-1})	Abundance ($10^9 l^{-1}$)	Bacterial production limitation[3]
TypAtoll 3																	
Haraiki	25	10	10	20	19	89	0.32	0.80	61	73	9.2	0.26	9.2	1.1	17.7	1.3	N
Hikueru	107	83	25	300	18	86	0.20	0.67	51	88	6.7	0.17	4.2	.7	9.2	0.9	N,P
Hiti	25	15	10	0	19	85	0.25	0.79	60	80	9.5	0.28	9.2	.9	13.9	2.4	C
Kauehi	343	315	50	200	22	88	0.15	0.47	29	76	7.2	0.17	4.2	.6	4.7	1.2	N
Marokau	256	217	30	50	17	73	0.21	0.61	41	63	8.0	0.31	7.5	.9	6.2	1.5	
Nihiru	100	80	20	20	25	75	0.13	0.73	56	63	6.9	0.30	5.0	.7	5.0	1.1	N
Reka-Reka	5*	1	1	0	2	88	0.43	1.43	144	100	12.7	0.47	18.3	3.9	102.6	1.4	P
Taiaro	17	12	12	3	1	57	0.32	2.58	201	159	15.3	0.21	15.0	1.7	17.0	1.8	N
Tekokota	7	5	3	0	59	48	0.03	0.45	28	63	7.6	0.19	4.2	.5	10.5	0.3	
Tepoto Sud	6	2	5	0	15	71	0.17	0.68	50	75	8.1	0.16	10.8	1.2	18.8	1.5	C
Tikehau	448	394	25	250	20	78	0.17	0.39	17	129			20.3	1.6			
Ocean						86	0.05	0.40	14	82	9.1	0.27	4.2	.6	0.8	0.5	C
TypAtoll 4																	
Haraiki								0.93	79	65	9.2	0.26	15.0	1.1	17.7	2.1	N
Hikueru								0.74	54	80	6.7	0.17	9.2	.7	9.2	0.9	N,P
Hiti								0.75	56	79	9.5	0.28	10.0	.9	13.9	1.7	C
Kauehi								0.55	35	71	7.2	0.17	7.5	.6	4.7	1.2	N
Marokau								0.65	45	73	8.0	0.31	9.2	.9	6.2	1.6	
Nihiru								0.71	52	62	6.9	0.30	9.2	.7	5.0	1.2	N
Reka-Reka								1.28	107	86	12.7	0.47	19.2	3.9	102.6	2.3	P
Taiaro								2.13	195	143	15.3	0.21	16.7	1.7	17.0	1.9	
Tekokota								0.43	28	62	7.6	0.19	7.5	.5	10.5	0.2	
Tepoto Sud								0.47	29	63	8.1	0.16	10.0	1.2	18.8	0.6	C,N
Ocean								0.37	14	86	9.1	0.27	4.2	.6	0.8	0.6	C,N

* The area of Reka-Reka atoll is given in error as 52 km^2 by Charpy et al. [1].

Conclusions

In conclusion, both seasonality and physical characteristics exert an influence on the composition of the microbial assemblages found in the water columns of atoll lagoons. A

dominant physical variable is exchange with the ocean which influences microbial community composition directly by wash-out and indirectly by its affect on the quantity and quality of dissolved organic matter, nutrient concentrations, etc.

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