



Application of denaturing gradient gel electrophoresis for detection of bacterial and yeast communities along a salinity gradient in the estuary of the Cachoeira River in Brazil

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Genet. Mol. Res. 12 (2): 1752-1760 (2013)

Received June 26, 2012

Accepted March 7, 2013

Published May 21, 2013

DOI <http://dx.doi.org/10.4238/2013.May.21.6>

ABSTRACT. An estuary is a transition zone between freshwater and marine ecosystems, resulting in dilution of seawater. Estuaries are also considered environments of intense biological activity related to the processes of nutrient cycling. The aim of this study was to evaluate the microbial community composition along a salinity gradient in the estuary of the Cachoeira River, located in southern Bahia, Brazil. The analysis of bacterial and yeast communities was performed by determining the denaturing gradient gel electrophoresis band richness. Formation of zones with similar profiles of bands was observed, and the increasing richness at the intermediate zone demonstrated a clear spatial distinction of communities depending

on salinity. In addition, the dissolved oxygen content, temperature, pH, salinity, and dissolved inorganic nutrient contents (NH_3^+ , NO_2^- , NO_3^- , PO_4^-) were determined. Nutrients were distributed in similar patterns, with decreasing concentrations as the salinity increases.

Key words: Microbial community; Estuary; Salinity; DGGE

INTRODUCTION

Estuarine ecosystems are crucial for maintaining marine diversity and form by interactions between the terrestrial environment and rivers and oceans. These ecosystems receive and concentrate material from drainage basins and a significant amount of organic and inorganic material from human activity (Tappin, 2002). As widely dynamic environments, the estuarine ecosystems are subject to considerable variation in the physicochemical parameters, and the microbial communities in estuary ecosystems are physiologically versatile. The estuarine bacterial community comprises autochthonous bacterial populations from freshwater and continental drainage (Divya et al., 2009). The distribution and abundance of estuarine microbial communities are influenced by biotic (competition, predation) and abiotic (salinity, temperature, dissolved oxygen) factors that modulate the behavior of the ecosystem in response to environmental changes (Fuhrman et al., 2006). This distribution is complex because of seasonal variations, mixing of water, and regular disturbances (such as changes in tide and input of nutrients, e.g., nitrogen) (Bernhard et al., 2005). Few studies have studied microbial communities in estuarine ecosystems in Brazil (Enrich-Prasta and Esteves, 1998; Abe et al., 2002; Filoso et al., 2006).

The estuary of the Cachoeira River is located in the city of Ilhéus, Bahia, Brazil ($14^\circ48'S$ to $39^\circ00'W$ and $14^\circ46'S$ to $39^\circ06'W$) (Figure 1). The Cachoeira River is approximately 65 km in length from its formation to its estuary in the city of Ilhéus (Klump et al., 2002). The estuary of the Cachoeira River is significantly contaminated by organic and inorganic materials from domestic sewage and industrial waste. There is also considerable local fishing activity, which renders investigations of this important environment. The objective of this study was to investigate the influence of the salinity gradient on the spatial distribution of microbial communities along the estuary using the denaturing gradient gel electrophoresis (DGGE).

MATERIAL AND METHODS

Water sampling

Sampling was conducted at 4 points, representing a salinity gradient of the estuary of the Cachoeira River (Figure 1). Point 1 is the marine station near Morro de Pernambuco, point 2 is located between the pier and the bridge of Pontefract, point 3 is located in the neighborhood of Vilela, and point 4 is located next to a sandbar. Samples were collected at the surface (in triplicate) using Van Dorn-type bottles, preserved in polyethylene bottles washed with 1:1 HCl/water, and the samples were maintained on ice until processing in the laboratory.

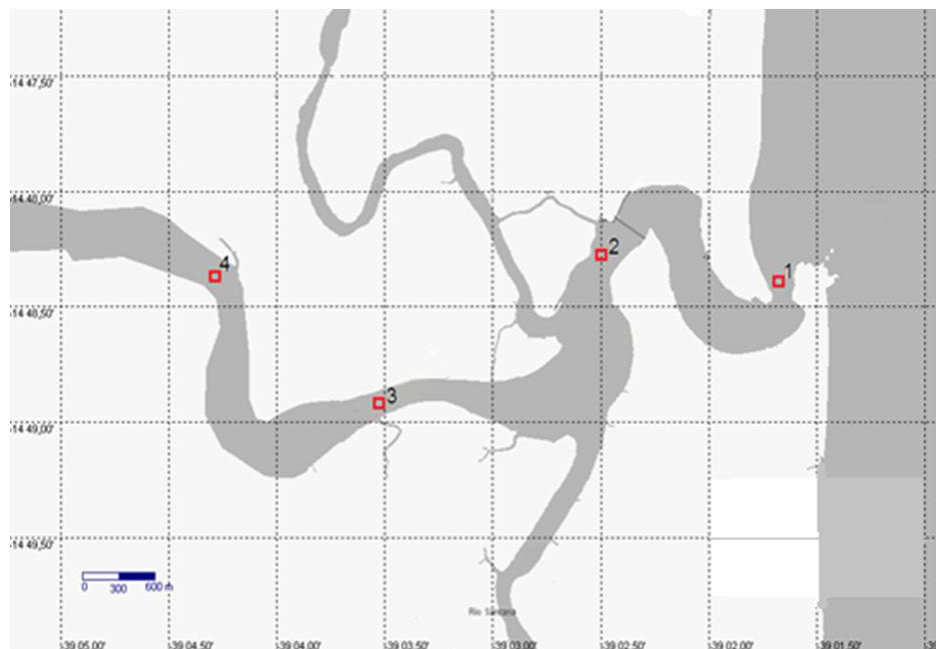


Figure 1. Location of sampling sites along the estuary of the Cachoeira River, Bahia, Ilhéus, Brazil. Point 1 = marine station near Morro de Pernambuco; point 2 = located between the pier and the bridge of Pontefract; point 3 = located in the neighborhood Vilela; point 4 = located after the sandbar in Vilela.

Physicochemical parameters

Salinity, temperature, and dissolved oxygen were measured in the field using a portable digital meter (Hanna model HI 93732N). In the laboratory, the samples were filtered through a Whatman GF/C 47-mm glass fiber (England), and the filtrate was frozen in polyethylene bottles. Ammonium, nitrite, nitrate, and phosphate (Sigma Aldrich) were measured spectrophotometrically (Thermo Scientific) in the visible region (Grasshoff et al., 1983).

DNA extraction

Aliquots of water (750 mL aliquots) were filtered through a 0.22- μ m membrane (Millipore). The membranes were sonicated (Ultrasonic Processor Model GEX-130 CE) in TE buffer (Tris-EDTA) for 20 s, and DNA was extracted with 25:24:1 phenol-chloroform-isoamyl alcohol (Sigma Aldrich). Five molar sodium acetate was used to remove proteins, and cold ethanol was used to precipitate nucleic acids. The DNA was washed with 70% ethanol, and the quality of the DNA was analyzed by 1% agarose gel electrophoresis (Sigma-Aldrich) (Henry et al., 2004).

DNA amplification and DGGE

DNA from yeast was amplified using primers for the 26S rRNA-encoding gene: NL1GC (5'-GCGCCATATCAATAAGCGGAGGAAAAG-3') and LS2 (5'-GCCATATCAATA

tal variation, showing a clear discrimination of point 4 compared with points 1, 2, and 3. This component represents a contrast between salinity and NO_2^- , NO_3^- , NH_4^+ , and PO_4^- . Hence, high NO_2^- , NO_3^- , NH_4^+ , and PO_4^- and low salinity shifted point 4 to the positive part of the graph, while high salinity and low NO_2^- , NO_3^- , NH_4^+ , and PO_4^- moved points 1, 2, and 3 to the negative part of the graph. Based on the similarity, the following groups exist: a) point 1, b) points 2 and 3, and c) point 4.

Table 1. Mean and standard deviation values for the data collected.

Sampling point	T°C	pH	Sal	O.D.	N- NH_4^+	NO_2^-	NO_3^-	PO_4^-
1	2.5 ± 0.15	6.13 ± 0.2	25 ± 0.28	8.45 ± 0.24	3.34 ± 0.36	1.02 ± 0.3	16.7 ± 2.17	3.83 ± 1.46
2	25.4 ± 0.17	6.3 ± 0	17 ± 0	7.62 ± 0.62	2.14 ± 0.95	1.0 ± 0.11	21.3 ± 0.98	3.58 ± 0.11
3	26.2 ± 0.25	6.43 ± 0.17	5.7 ± 0.28	7.94 ± 1.0	3.04 ± 0.95	1.5 ± 0.17	34.0 ± 3.99	6.22 ± 0.75
4	26.1 ± 0.15	7 ± 0.15	3.16 ± 0.28	8.21 ± 0.2	7.33 ± 2.3	1.76 ± 0.02	44.7 ± 0.5	8.9 ± 0.17

Sal = salinity; O.D. = oxygen dissolved.

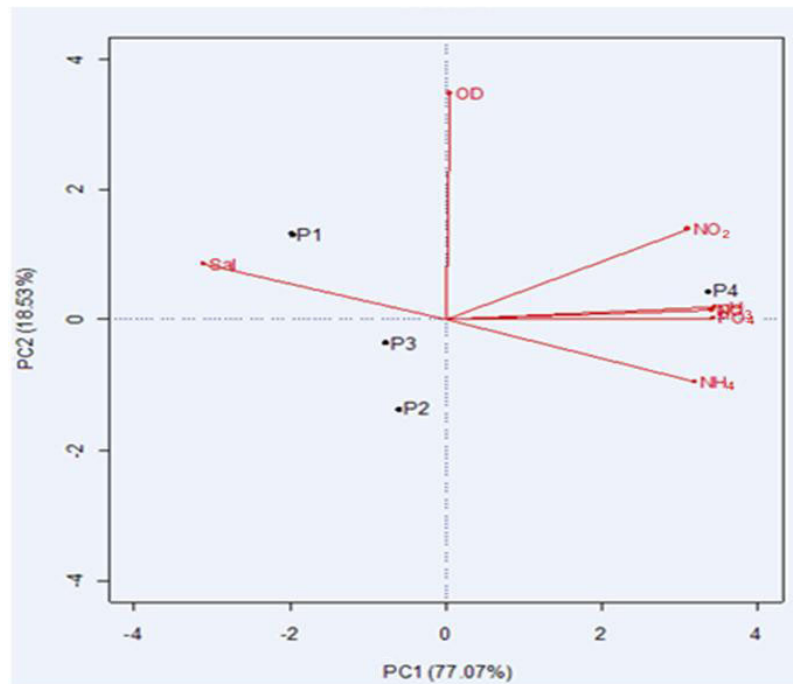


Figure 2. Biplot of principle components (PCs).

Microbial diversity

Banding patterns observed by DGGE were used to estimate the community structure of microorganisms in the estuary. This estimation was performed by cluster analysis of DGGE bands observed for each sample (Figures 3 and 4).

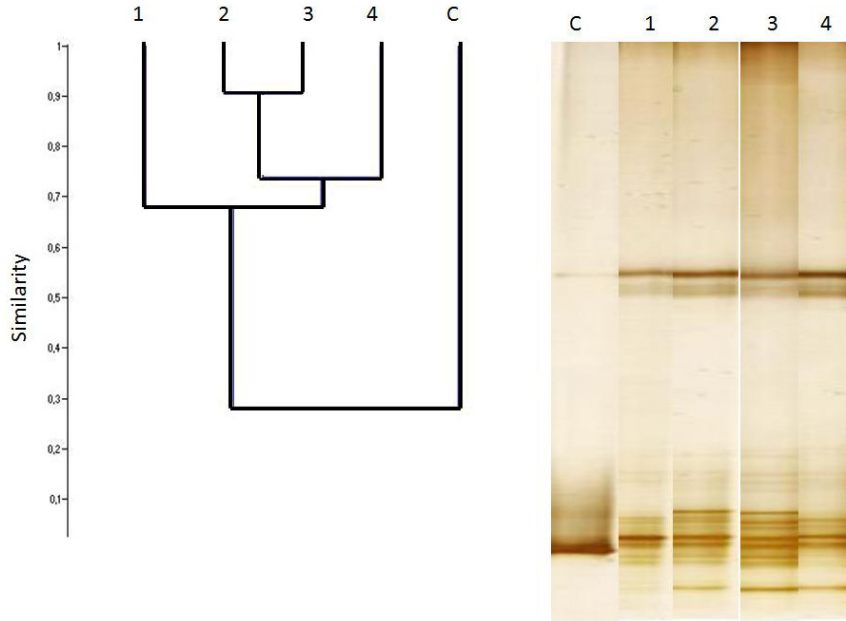


Figure 3. Dice's coefficient of similarity and dendrogram generated from the denaturing gradient gel electrophoresis band profile of the amplified fragment with primer 16S (for bacteria). For definition of points 1-4, see legend to Figure 1. C = control.

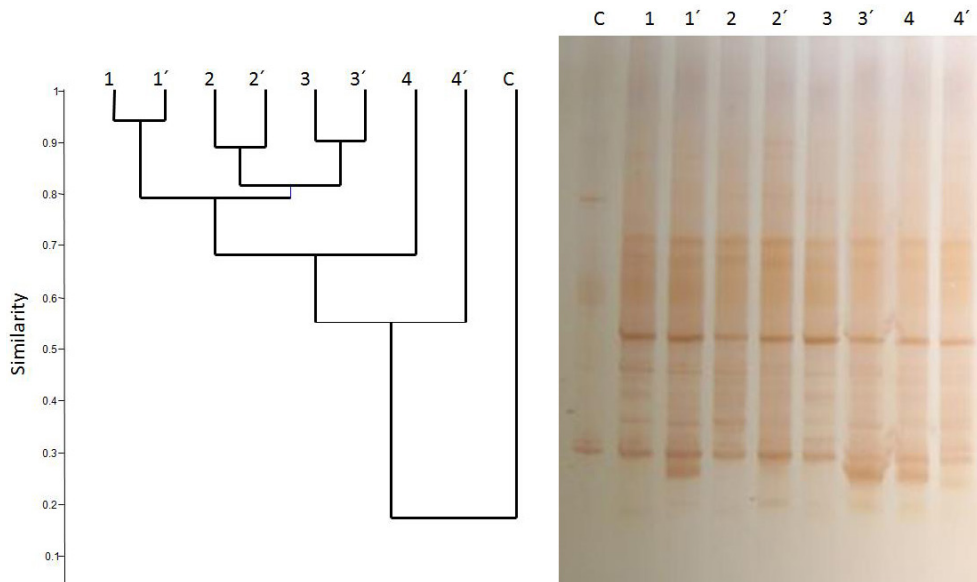


Figure 4. Dice's coefficient of similarity and dendrogram generated from the denaturing gradient gel electrophoresis band profile of the amplified fragment with primer 26S (for yeast). Samples 1, 2, 3, and 4 are in duplicate. For definition of points 1-4, see legend to Figure 1. C = control.

The dendrogram generated based on the DGGE profile of 16S rDNA (bacteria) showed that communities at points 2 and 3 were 90% similar. Similar to what was observed for the bacterial communities, DGGE analysis showed that the yeast communities at points 2 and 3 (inner regions of the estuary) were similar (Figure 5). The Venn diagram shows grouping of bands that may be shared between sample points or unique to sample points. Based on Figure 5, a greater number of bands are shared between points 2 and 3 in the bacterial DGGE analysis. These 2 sampling points also shared 2 unique bands (not present at points 1 and 4), thus demonstrating the similarity of species distribution, corroborating the profile generated by biplot and PC analyses. The sampled points can be regarded as an intermediate zone along the salinity gradient, with a particular degree of microbial diversity. The DGGE profiles of bacteria suggested greater species richness at points 2 and 3, and both gels contained 17 bands. Points 1 and 4 had lower salinity and gave 14 bands and 13 bands, respectively. In the yeast DGGE analysis, points 1, 2, and 3 had high salinity and the corresponding DGGE contained 8 bands; point 4 had lower salinity and the gel contained 6 bands, suggesting less yeast community diversity at point 4 compared with points 1, 2, and 3. Ten 16S DGGE bands were observed at all points along the gradient, indicating that some microorganisms are maintained throughout the gradient. In addition, points 2 and 3 presented 16 bands in common and 2 unique bands, consistent with the similarity of 90% obtained in the dendrogram.

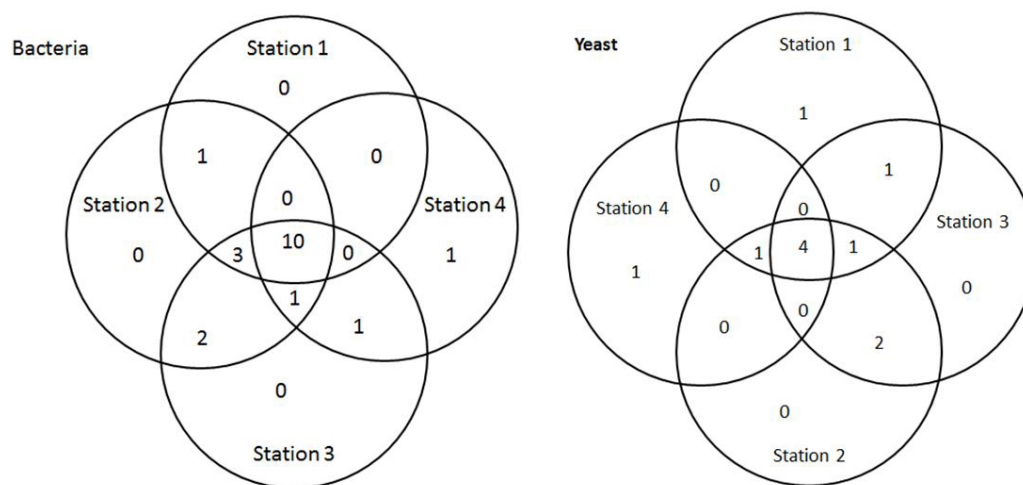


Figure 5. Venn diagram of the denaturing gradient gel electrophoresis band profiles at the different sampling points.

DISCUSSION

The high oxygen values suggest high phytoplankton activity. The photosynthesis of algae occurs largely in eutrophic waters, where the decomposition of organic materials causes release of minerals, especially those containing nitrogen and phosphorus (Braile et al., 1993). The low concentration of nitrite can be explained because it is an intermediate phase nutrient between ammonium and nitrate. High nitrite values can be found in the water

output of domestic sewage and is considered an indicator of organic pollution (Baumgarten et al., 2001). High levels of nitrite in the water also correspond to high bacterial activity and lack of oxygen. The values for nitrate can be explained by continental drainage because point 4 is located in the inner region of the estuary. Other studies have reported that nitrate is the predominant dissolved inorganic nutrient in estuary systems (Tundisi et al., 1991; Boyer et al., 2002). A point source directly influenced by waste generated by nearby communities may be responsible for the high phosphate content. However, many natural processes contribute to the release of phosphorus in the water column, such as senescence of phytoplankton that releases dissolved organic phosphorus, microbial activity, and zooplankton that regenerates phosphorus in the water column, and excretion of organisms, where the phosphate released is consumed by primary producers. Furthermore, the river flow carries phosphate from the decomposition of terrestrial plants, animal waste, and human and industrial sewage (Herlemann et al., 2011).

In relation to microbial analyses, the high band similarity between points 2 and 3 can be explained by the analogous physicochemical properties of these points. Point 4 was less similar compared with the other sampled points, what can be explained by the lower salinity of this point (Figure 2). The microbial distribution in the estuary is complex because of seasonality and variations in the brackishness of the water, in addition to regular disturbances such as changes in tides and nutrient levels. However, salinity is the main environmental factor in the distribution of organisms, regulating habitats and microbial diversity (Tundisi et al., 1991; Herlemann et al., 2011). Yeast is used as an indicator of environmental quality (Peçanha et al., 1996). The presence of yeast in estuarine water samples signifies an eutrophic environment because of high concentrations of organic matter.

DGGE analysis confirmed the presence of various microbial groups in all sampling points based on amplification with primer 16S. Although point 4 is associated with a greater degree of pollution, it also features greater microbial diversity. The physicochemical properties support the distribution of microbes but do not explain why some bands are absent in the DGGE profiles. This absence of certain microbial groups can also be attributed to differences in salinity. Recent studies have shown that salinity can directly affect the distribution of some groups of microorganisms (Herlemann et al., 2011).

The results found for yeasts corroborate with the profile found for the distribution of bacterial communities, suggesting that the physicochemical variables directly influenced the distribution of the microbiota along the estuary. Some microorganisms are likely favored at the ends of the salinity gradient because some bands are only observed at points 1 and 4. The DGGE of yeast and the single band at point 4 are indicative of lower salinity. However, we found an abundance of bacterial species in the intermediate regions of the estuary. DGGE of yeast rDNA appears more static with increasing levels of salinity. Therefore, we suggest that there is a spatial stratification, with 3 zones with different properties and microbial communities.

CONCLUSION

The data showed a higher microbial richness in areas with intermediate salinity and different levels of pollution. DGGE revealed a slight decrease in species richness as the salinity decreased. Point 4 showed a small number of bands and higher levels of nitrate and phosphate, showing a selective pressure along the salinity gradient.

ACKNOWLEDGMENTS

Research supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Process #558272/2009-6) and Universidade Estadual de Santa Cruz.

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