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Ecology and Diversity of Marine Microorganisms ECODIM IV

MICROBIAL DIVERSITY IN BIOFILMS FROM THE INTERTIDAL ZONE IN COLIUMO BAY, CHILE

Ivan Calderon[1], M. Alexandra Garcia[2], Marcelo Gutierrez[3], Gerdhard Jessen[4], Jennyfer Mora[5], Juan Ugalde[6], Osvaldo Ulloa[3,8] & Kurt Hanselmann[7,8]
[1]Universidad de Santiago de Chile, [2]Instituto Venezolano de Investigaciones científicas, [3]Universidad de Concepción, [4]Centro FONDAP-COPAS, [5]Universidad Nacional de Colombia-INVEMAR, [6]Universidad de Chile, [7]Universidad de Zurich, Suiza, [8]Course coordinator.

The microbial diversity in biofilms which develop in the intertidal zone was analyzed using microscopy and molecular approaches. Samples were collected from different substrates (rocks, shells, plant surfaces etc.). The observation of the samples using epifluorescence microscopy indicated the presence of microorganisms in all of them. DNA was isolated and amplified with universal eubacterial primers for the 16s rRNA gene. Positive results were obtained from the following substrates: algae from the low and the high intertidal level, wood at the low intertidal level and estuarine plants. RFLP analyses revealed differences in the community composition between the substrates from which the biofilms originated. Differences were observed between epiphytic biofilms from low and high intertidal levels. No differences were observed between algal surfaces and wood from the low intertidal level. Our results suggest that there is a zonation of microbial communities in biofilms of intertidal estuarine zones which seems to depend less on the type of substrate but more on salinity and intermittent emergence and flooding as determinants.

INTRODUCTION

A biofilm is an assemblage of microbial cells that are associated with a surface and enclosed in a matrix of exopolymers. In a natural aquatic system, biofilms are highly complex structures that may include clay and sand minerals, diatoms and different kinds of bacteria (Donian, 2002). Within hours an organic film may form over a submersed surface which can facilitate the adhesion, colonization and growth of microbial populations from the water column (Costerton *et al.* 1987, Jackson *et al.* 2001). The abundance and diversity of microorganisms in these biofilm communties is regulated by physico-chemical factors such as emergence stress, salinity, oxygen and nutrient availabilty and biological factors such as grazing.
The application of molecular techniques to microbial community analysis has facilitated more complete examinations of microbial assemblages with an ecological perspective (Jackson *et al*, 2001). In this study we utilized RFLP pattern analysis to describe the differences in biofilm community structures on different substrates in freshwater and marine salt water.

OBJECTIVES

The aim of this study was

- to compare the microbial diversity in biofilms from different substrates and
- to determine the effect of the intertidal zonation on the microbial diversity present in biofilms

MATERIAL AND METHODS

SAMPLING

Samples from different substrates and from different intertidal zones were collected along the coast of Dichato beach on January 4, 2006. The substrates were collected in high, middle and low intertidal zones and from abiotic and biotic substrates. The abiotic substrates were shells, rock, concrete and wood from the marine environment while the biotic substrates were algae, mollusks (feces) and a plant from a freshwater environment.
In addition, glass microscope slides were chosen as a uniform surface for biofilm development. Arrays of slides were exposed at two intertidal levels (high and low zones) and were collected after 2 days.

ANALYSES

Epifluoresce microscopy.
DAPI stain was used to determine cell abudances in biofilms which had grown on the slides in the marine environment. The cells were counted with the aid of an epifluorescence microscope (Axiolab MC80, Zeiss) in ten different fields (Figure 1.) In all samples the presence of cells could be detected by DAPI stain and epifluorescence microscopy (Figure 2).

Molecular techniques:
DNA extraction by the phenol-chloroform method and 16S rDNA PCR amplfication with universal primers for eubacteria (8F, 1392R) was carried out with all samples. The DNA and PCR products were run on an electrophoresis gel using 1% agarose, stained with ethidium bromide and visualized under UV light.
RFLP analysis of 16S rDNA fragments of amplification products were digested with the restriction enzyme *AluI* for 12 hours at 37°C. The digestion products were separated by electrophoresis in 3% agarose gels.

STUDY SITE

Coliumo Bay in Central Chile is the largest (167.4 km^2), and most enclosed embayment along the Chilean coast

RESULTS

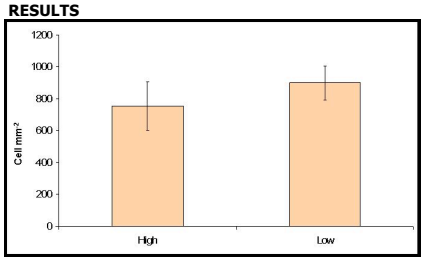

Figure 1. Cell abundance on glass slides at high and low points in the intertidal zone. Error bars reflect standard deviations of ten parallel measurements

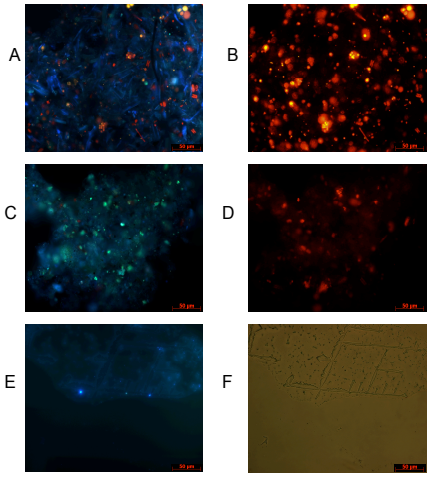

Figure 2. Epifluorescence of DAPI-stained samples from different substrates. *A*, wood (UV light). *B*, wood (emission 546 nm). *C*, rock (UV light). *D*, rock (emission 546 nm.). *E*, gastropod feces (UV light). *F*, gastropod feces (phase contrast). Scale bars represents 50 µm.

Sample	Substrate	Intertidal Level	DNA	PCR	RFLP
1	Algae	Low	+	+	
2	Wood	Low	+	+	+
3	Shell	High	-	-	
4	Iron Column	High	-	-	
5	Rock	High	-	-	
6	Concrete	High	-	+	-
7	Algae	Middle	+	+	
8	Brown Algae	High	+	+	+
9	Rock	Low	-	-	
10	Wood	High	-	-	
11	Brown Algae	Low	+	+	+
12	Rock	Middle	-	-	
13	Rock	High	-	-	
14	Rock	Low	+	-	
15	Shell	High	+	-	
16	Rock	Low	+	-	
17	Algae	High	-	-	
18	Green Algae	High	+	-	
19	Shell	Low	+	-	
20	Rock	High	+	-	
21	Green Algae	High	+	-	
22	Shell	Middle	+	-	
23	Shell	High	+	-	
P	Plant		-	+	+
G	Rock		+	-	-
A1	Gastropod Tissue		-		
A2	Bivalve Tissue		-		
A3	Gastropod Tissue		-		
A4	Bivalve Tissue		+	-	
B1	Gastropod Feces		+	+	-
G1	Bivalve Feces		+	+	-

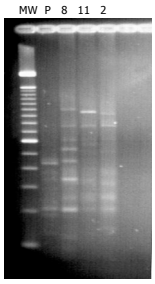

Table 1(left) RFLP and PCR results obtained from different substrates in three intertidial zones
Figure 3 (right) RFLP profiles of *AluI* digested 16S rDNAs amplfication products from 3 substrates of 2 intertidal levels. *P*: Plant (fresh water). *8*: Brown algae (high level). *11*: Brown algae (low level), *2*: Wood (low level). Samples 8, 11 and 2 are from salt water environments.

DISCUSSION

When extracting DNA from environmental samples it is important to consider the presence of inhibitors that may affect further analyses. "Cleaning" of the DNA with CTAB (cetyl-trimethylammonium bromide) is recommended for DNA extractions from environmental samples (Murray and Thompson, 1980. Holben *et al.* 1988), especially when the environmental sample comes from an inorganic surface or if it contains a high content of organic matter.
Our RFLP analysis (Figure 3), illustrates the differences in the composition of the microbial communities between substrates obtained from seawater and from estuarine zones, confirming that environmental variables (like salinity), affect the conditioning of surfaces and, as a consequence, the microbial diversity in the biofilm. We observed differences between epiphytic biofilms from algae growing at low and high intertidal levels. However, no differences were observed between biofilm patterns from algae or wood as substrate at the low intertidal level. These results suggest that the zonation of microorganisms in biofilms is regulated more strongly by the environment rather than by the type of substrate. This type of zonation is known to exist at the macroscopic level in the intertidal zone (Santelices, 1998). Our results suggest that this regulation also applies to microbial biofilms. Environmental variables like solar radiation, oxygen, temperature and the cyclic presence and absence of water may regulate the formation of biofilms and its diversity more strongly than the type of substrate.
Epifluorescence microscopy confirmed the presence of certain types of microorganisms (e.g. bacteria, cyanobacteria) and the RFLP demonstrated the microbial species richness. This was reported by other authors as well (Davey and O'toole, 2000. Nocker *et al*, 2004), but the interactions between the bacterial communities and between them and the environmental determinants are still open questions.

CONCLUSIONS

Our results obtained by RFLP suggest that a biofilm zonation exists in the intertidal zone of Coliumo Bay which is independent of the type of substrate. Environmental variables like salinity and tidal emergence might be the essential factors affecting the microbial diversity in biofilms of these estuarine and sea water habitats.

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