



# A Study of Bacterial Communities Associated with Podostemaceae Biofilms

Department of Biological and Environmental Sciences; Western CT State University

Kristy Garren and Ruth A. Gyure

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## ABSTRACT

The focus of this study is characterization of bacterial communities associated with members of the plant family Podostemaceae. *Cyanobacteria* (blue green bacteria) play a major role in the secretion of adhesive compounds that allow these plants to attach to rocks in waterfalls, and thus survive rapid flowing water and rapidly changing environmental conditions. For this study, several isolates were cultured from biofilm tissue and information about the community was obtained utilizing PCR (polymerase chain reaction) to show presence or absence of important groups (taxa) of bacteria.



## RESULTS AND DISCUSSION

1. 16s results confirm that bacterial DNA is present in samples from all sites (Figure 1). We then used group specific primers to look at major bacterial taxa in the biofilm layer. The 16S product also served as template in further PCR amplifications when original amplicon yield was low.

2. Cyanobacterial DNA was amplified from each site (Figure 2). These are perhaps the most important group of bacteria in the biofilm and results support those obtained by Jäger-Zürn *et al.* (2000) who first proposed that they produce the sticky substance that allows the Podostemaceae to attach to the substratum. The Cyanobacteria finding is also significant because this group has the ability to photosynthesize as well as to fix nitrogen. Because the rivers where Podostemaceae are found are low in nutrients, this gives us some insight as to how they can survive.

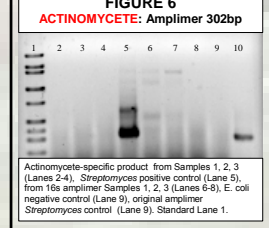
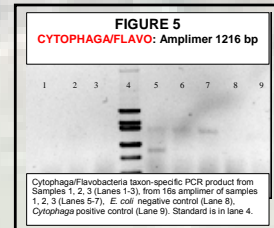
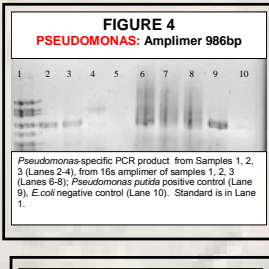
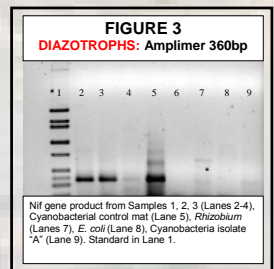
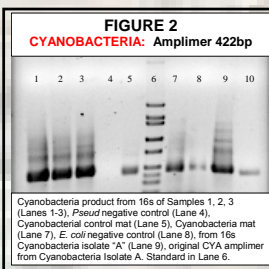
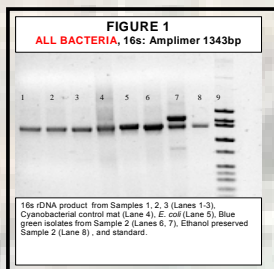
3. The genes necessary for nitrogen fixation are also found in bacteria called diazotrophs and were amplified from sites 1 and 2 (Figure 3) and also from Cyanobacteria positive controls. Non-cyanobacterial nitrogen fixing bacteria are present in freshwater, seawater, and soil and are clearly important for the capture and cycling of nitrogen in these ecosystems.

4. *Pseudomonas* was found in two of the biofilm samples (Figure 4). Members of this genus are known to form close associations with plant roots, produce exopolysaccharides (*slime layers*) and also have the ability to metabolize a variety of nutrients.

5. Populations of cytophaga/flavobacteria are often found in diverse environments ranging from Antarctic sea ice to terrestrial soils and previous studies report finding them in epilithic biofilms. These bacteria have the ability to break down macromolecules such as cellulose, agar, and chitin, which makes them of considerable practical importance. Amplification with Cytophaga/Flavobacteria-specific primers gave poor yield of amplicon, but there does appear to be a positive result for Site 3 (Figure 5).

6. Actinomycetes are usually found in the soil, and include some important decomposers of organic materials such as cellulose and chitin. Actinomycete-specific primers yielded no amplicon from any of the 3 sites, which was surprising (Figure 6).

7. Ammonia oxidizers (*Nitrobracter* sp.) are important bacteria of the nitrogen cycle, but would not be expected in a "low nutrient environment" such as this biofilm. Our primer results show no amplification from the study sites (Figure 7) with strong positive results in the controls.



Primer	Sequence	Reference	Group/Taxon
BAC28F BAC436R	5'-GGGGGTCATACACATGCAAGTC-3' 5'-AGCGGCGGCGTGTGATG-3'	Lane, 1991	All Bacteria
CYAN58F CYAN76R	5'-GG GGA ATC TTC CCG AAT GGG-3' 5'-GAG TAC TGG GGT ATC TAA TCC CAT T-3'	Nebel, 1997	Cyanobacteria
ACT228F ACT228R	5'-GGA TGA GGC CCG GGC CTA-3' 5'-GG GGC GCG GCT GCT GGC AGC TA-3'	Heuer, 1997	Actinomycetes
PSI PSI	5'-GGG TCT GAG AGG ATG ATG AGT-3' 5'-TTA GCT CGA CCT GGC GGC-3'	Wolner, 1998	Pseudomonas
Amn18F Amn28R	5'-GGGAGCTGGGACTCTTG-3' 5'-GAGGCGGAGGAGGAGG-3'	Holmes, 1995	Ammonia oxidizers
Nit229F Nit229R	5'-TCT TTT GAG ATT TGT CAG-3' 5'-GCT AAA ACT CAA AGG AAT TGA-3'	DeGraaf, 1995	Nitrate oxidizers
CF319F Eub1520R	5'-CTG GTA CTG AGA CAC GGA-3' 5'-AAG GAG GTG ATC CAN CQR CA-3'	Kinchin, 2003	Cytophaga, Flavobacteria
Phal Phal	5'-TGC GAY CDS AAR GGB GAG TC-3' 5'-ATG GGCATCATY TOR CCG CA-3'	Poly, 2001	Diazotrophs

## MATERIALS AND METHODS:

**Specimen Collection:** The specimens used in this study were collected by Philbrick, Bove, and Tucci, July 2006 from 3 locations in Brazil:

- 1) Sample 1 from São João do Araguaia, submerged flowering plants
- 2) Sample 2 from São João do Araguaia, flowering plants attached to rocks at water's edge
- 3) Sample 3 from Taquarussu, outcrop, no flowering plants.

Rocks were stored either DRY (in sterile paper) or in absolute ethanol for transport back to WCSU laboratory (maximum transport time 7 days). Once at the laboratory, material was scraped from rock surface for DNA extraction. Dry material on rock was first ethanol sterilized (by 1 minute soak in 100% ethanol) then re-dried, to remove any external non-attached bacteria before biofilm DNA extraction. Material was scraped from rock using sterile forceps and scalpel.

**DNA extraction:** Approximately 0.5g of the scraped material was used for extraction with Mo Bio Powersoil™ Kit. This kit extracts genomic DNA using a bead beating mechanical and chemical lysis, and silica membrane capture and elution of purified DNA. Purified DNA was stored at -20°C until used for polymerase chain reaction (PCR) and/or cloning.

**PCR:** Taxon-specific primers were used to specifically amplify either the 16s rRNA gene, or other targets (Table 1). For all PCR reactions, reaction mix included 1.25 U GoTaq® DNA polymerase (Invitrogen), 1X reaction buffer with 1.5mM MgCl2, 0.2mM each dNTP, and approximately 50ng DNA template. Thermocycling program: Preheat at 94°C, 30 cycles of 94°C for 30s, 55°C for 30sec and 72°C for 30sec, and final extension at 72°C for 10 minutes. Amplicons were purified using QiaQuick™ Spin Columns and viewed on 0.2% agarose gels.

## REFERENCES:

- Jäger-Zürn, I., and M. Grubert. 2000. Podostemaceae depend on sticky biofilms with respect to attachment to rocks in waterfalls. *Int. J. Plant Sci.* 161(4): 599-607.
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