# COMPARISON OF BIOFILM MICROBIAL COMMUNITIES DEVELOPED IN IRRIGATION SYSTEMS USING TREATED WASTEWATER FROM DIFFERENT WASTEWATER TREATMENT PLANTS (WWTP)

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## Abstract

In order to address water shortage, reuse of treated wastewater or utilization of agronomic waters has been long proposed and used as a viable option for irrigation. Utilization of treated wastewater for irrigation rises a number of issues related both to the safety of the process, as well as to the appearance of hydraulic problems due to the formation of microbial biofilms. The aim of this work was the molecular characterization through a fingerprinting technique (DGGE, Denaturing Gradient Gel Electrophoresis) of microbial communities in biofilms from irrigation systems using treated wastewater from different wastewater treatment plants (WWTP) of two Spain locations (Almería and Gran Canaria) and their comparison. The fingerprints obtained from Almería biofilms showed a reduced number of bands, indicating that diversity was low; sequences obtained from DGGE bands corresponded to esporulated and thermophilic members of the Firmicutes group, suggesting a selective role of temperature in this kind of system. On the other hand, DGGE patterns obtained from Gran Canaria biofilms showed the presence of a large number of bands, indicating that the microbial community was rather complex.
Key words: DGGE, wastewater reuse, biofilms

## Introduction:

The traditional scarcity of hydric resources in Spain linked to a growing trend in water use for applications such landscaping, gardening or agricultural irrigation is gradually shifting water management practices towards the use of regenerated water obtained from wastewater treatment plants. The use of properly conditioned regenerated water is safe from a sanitary point of view but some concerns have been raised regarding the effects generated by the continuous use of this type of water in distribution networks. Most of the problems arise from biofilm formation at the inner surfaces as well as at the points of delivery, and consequently, the study of the composition of these communities becomes crucial.

The aim of this work was the molecular characterization of microbial communities in biofilms from irrigation systems using treated wastewater from different wastewater treatment plants (WWTPs) of two Spanish locations (Almería and Gran Canaria), as well as the comparison between both assemblages.

## Methods:

*Sampling*: Biofilms from the inner plastic surfaces of two irrigation systems, which used treated wastewater from WWTPs of two Spanish locations (Almería and Gran Canaría) were scraped and stored at −20°C until use.

*DNA extraction*. Upon thawing, the total community DNA was extracted using the DNA Power Soil kit from MOBIO (12888-50).

*PCR-DGGE fingerprinting*. The 16S rRNA genes suitable for DGGE (Denaturing Gradient Gel Electrophoresis) analysis were amplified by polymerase chain reaction (PCR) using the specific primer set 358f-907rM (Sánchez et al. 2007). PCR was carried out with a Biometra termocycler using the program and conditions described by Sánchez et al. (2007).

DGGEs were run in a DCode system (Bio-Rad) as described by Muyzer *et al*. (1998). A 6% polyacrylamide gel with a gradient of 30-70% DNA-denaturant agent was cast by mixing solutions of 0% and 80% denaturant agent (100% denaturant agent is 7 M urea and 40% deionized formamide). Seven hundred ng of PCR product were loaded for each sample and the gels were run at 100 V for 18 h at 60°C in 1xTAE buffer (40 mM Tris [pH 7.4], 20 mM sodium acetate, 1 mM EDTA). The gel was stained with SybrGold (Molecular Probes) for 45 min, rinsed with 1xTAE bufer, removed from the glass plate to a UV-transparent gel scoop, and visualized with UV with a Chemi Doc XRS (Bio-Rad). Prominent bands were excised from the gels, resuspended in milli-q water overnight and reamplified for their sequencing.

Purification of PCR products and sequencing reactions from DGGE bands were performed by Macrogen (South Korea) with primer 907rM. Macrogen utilized the Big Dye Terminator version 3.1 sequencing kit and reactions were run in an automatic ABI 3730XL Analyzer-96 capillary type.

Sequences were subjected to a BLAST search (Altschul *et al*. 1997) to obtain an indication of their phylogenetic affiliation.

## Results and Discussion:

The fingerprinting obtained from Almería irrigation system biofilms (see Fig 1A) showed a reduced number of bands, indicating that diversity was low. Only two taxonomic groups, Firmicutes and γ-proteobacteria, could be found. Most of these sequences belonged to cultured bacteria. Virtually all the retrieved bands had a high similarity with esporulated and thermophilic members of the phylogenetic group Firmicutes (Gram positives with low G+C content), suggesting that only microorganisms able to grow at high temperatures were selected.

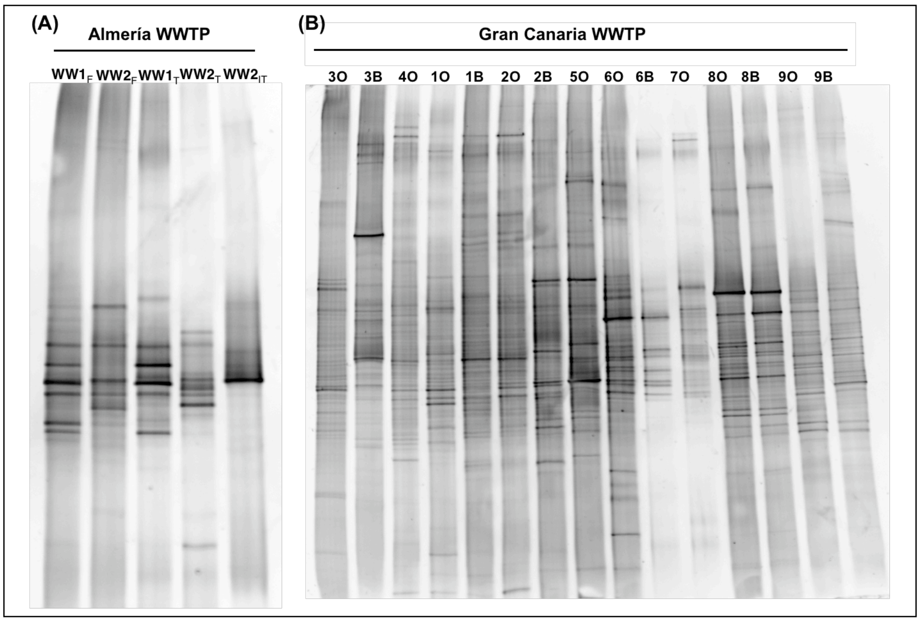


***Figure 1: (A)** DGGE fingerprints of Almería's biofilms. WW1$_F$: biofilm from tap wastewater 1 filter. WW2$_F$: biofilm from wastewater 2 filter. WW1$_T$: outer biofilm from tap wastewater 1 pipe. WW2$_T$: outer biofilm from wastewater 2 pipe. WW2$_{IT}$: inner biofilm from wastewater 2 pipe. **(B):** DGGE fingerprints of Gran Canaria's biofilms. The numbers indicate different drippers. O samples: biofilm from drippers sampled before pressure cleaning of the sistem. B samples: biofilm from drippers sampled after pressure cleaning of the sistem.*

On the other hand, DGGE patterns obtained from Gran Canaria biofilms (see Fig 1B) showed the presence of a large number of bands, indicating that the microbial community was rather complex. Most of the retrieved DGGE bands belonged to the phylogenetic groups γ-proteobacteria, Nitrospirae, β-proteobacteria, Bacteroidetes, Chlorobi, Acidobacteria, Actinobacteria, Firmicutes and Chloroflexi, but sequences from Deinococcus-Thermus, δ-proteobacteria, Gemmatimonadetes and Planctomycetes were also found. The results showed that the Gran Canaria biofilm had a heterogeneous composition with representation of groups usually found in aquatic environments. Most of these sequences belonged to uncultured microorganisms, while others matched well with cultured bacteria. The similarities ranged between 81.3 and 100%. Some of the retrieved bands had a high similarity with genus involved in the nitrogen cycle, like *Nitrospira* sp. (Nitrospirae) which is a nitrite-oxidizing bacterium often found in wastewater treatment systems (Hovanec *et al*., 1998), or *Bradyrhizobium* sp. (α-proteobacteria), a genus of Gram-negative soil bacteria with many species able to fix nitrogen. Other bands of cultured bacteria belonged to microorganisms involved in biofilm formation, like *Acinetobacter* sp. or *Sphingomonas* sp., widely distributed in nature. In drinking water, *Acinetobacter* have been shown to contribute to bacterial aggregation while *Sphingomonas* sp. is a genus recognized by its capability to degrade a wide variety of refractory environmental pollutants and carry out diverse other biotechnologically useful activities, such as the biosynthesis of valuable biopolymers (Laskin and White, 1999). *Sphingomonas* sp. could also be involved in biofilm formation due to its ability to form exopolimers associated with the initial adhesion of bacteria, which is the primary step for biofilm formation (Azeredo and Oliveira, 2000).

## Conclusions:

The microbial communities of biofilms developing in irrigation systems using treated wastewater depends on several environmental factors like temperature or wastewater origin. In Almeria's biofilms, it was observed a selection due to high temperatures towards a low diversity assemblage of esporulated and thermophilic members of the Firmicutes group. In Gran Canaria a more complex microbial community developed DNA-based molecular tools showed that these biofilms contain significant hidden diversity of unknown and uncultured microorganisms.

Acknowledgements: This work was supported by grants CSD2006-00044-TRAGUA and CTQ2009-14390-C02-02 from the Spanish Government to J. Mas.

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