Evolution of the biodiversity and interspecific relationships in river biofilms from a preserved environment or exposed to pharmaceuticals

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Context and Aim

Microorganisms forming river biofilms, when exposed to various pollutants, could have their taxonomic composition impacted as formerly described in several publications. It could be the case of the ones exposed to the effluent of a pharmaceutical factory WWTP (wastewater treatment plant), which was previously associated with an abnormal development of fish gonads and subsequently reinforced by new filtration systems to avoid this problem with success. The aim of this study is to analyse the impact of this effluent on the evolution of the biodiversity and interspecific relationships in downstream river biofilms during six months. Different supports for biofilm development and several primer sets were tested in order to describe the microbiological diversity with the most exhaustive manner, and to detect potential perturbation of the microbiota even in presence of an effluent weakly loaded in xenobiotics.

Methods

The WWTP treated effluents of a factory producing chemicals. The biofilm collectors used were glass plates and low density polyethylene (LDPE) membranes. Biofilm collectors were placed in the mid-mountain river (average annual flow about 20m³/s), upstream and downstream the WWTP effluent, near to chemicals collectors. Some physico-chemical parameters were measured every day. After one month, the concentration of 7 pharmaceutical molecules were determined and biofilms were harvested. After DNA extraction, 16S and 18S rDNA fragments were amplified and sequenced (MiSeq, Illumina). Sequences were processed as described in the UPARSE pipeline to generate OTUs (operational taxonomic unit). QIIME and R were used for statistical analyses. GraphViz was used to build microbial interaction networks.

Results

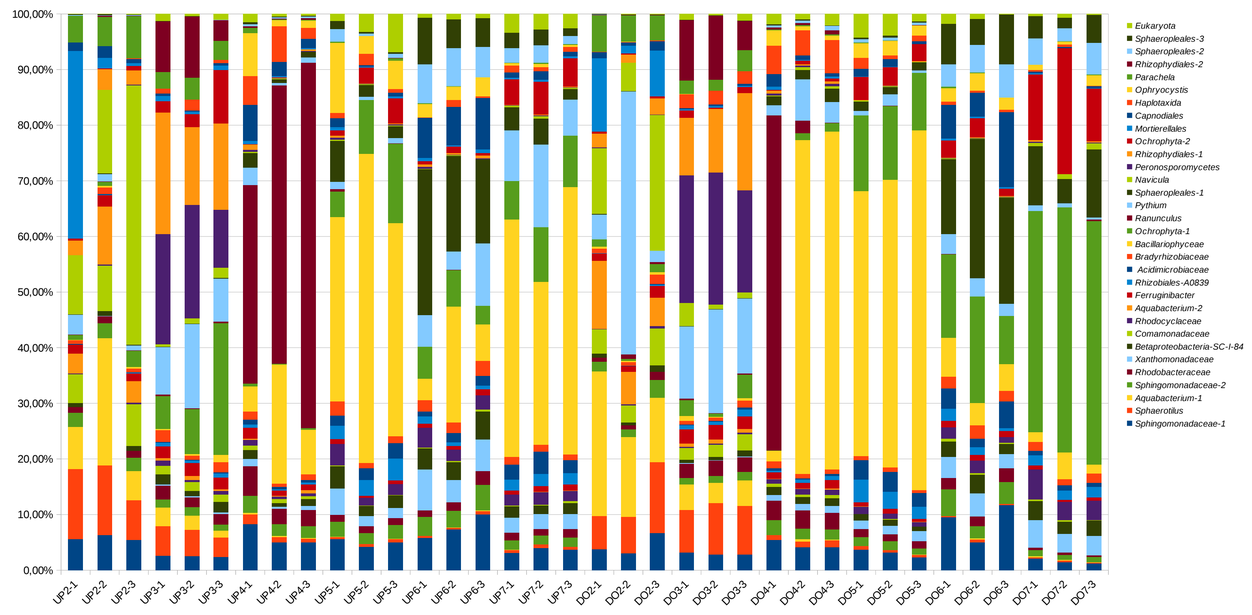

Figure 1: Relative abundance (RA) of OTUs belonging to the major core microbiota. For each month (2: March to 7: August), 3 samples were harvested (-1 to -3) upstream (UP) and downstream (DO) the WWTP effluent. According to the non-parametric t-test used, there was no OTU significantly more or less abundant upstream or downstream the WWTP effluent.

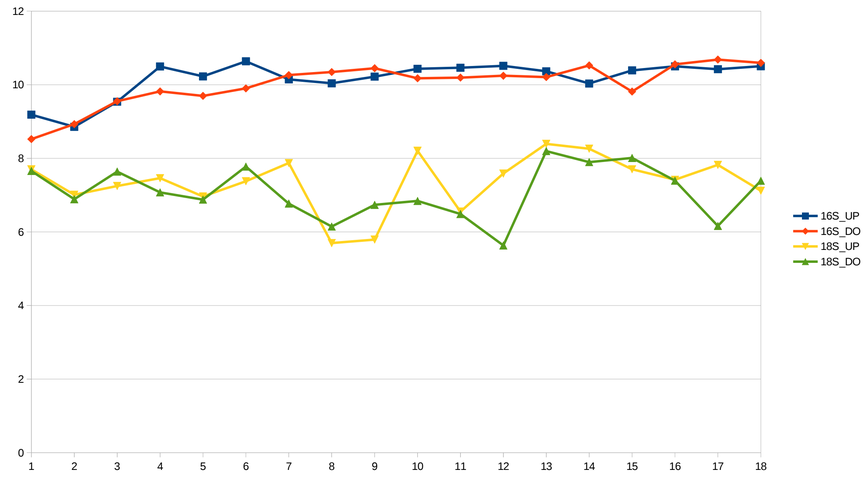

Figure 2: Shannon index of diversity calculated on prokaryotic and eukaryotic communities upstream and downstream the WWTP effluent. The diversity of Prokaryota (16S) is more important than the diversity of Eukaryota (18S), but in each case, there is no significant difference between samples (1 to 18) from upstream (UP) and downstream (DO) the WWTP effluent.

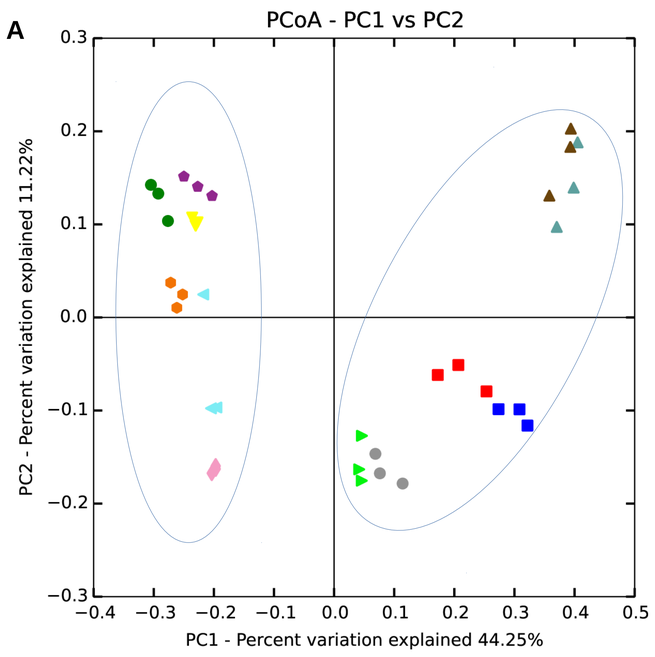

Figure 3: Principal coordinates analysis (PCoA) of biofilm samples according to the sampling place and microbial community composition and abundance. A) PCoA of prokaryotic community structure based on a Bray Curtis dissimilarity matrix. PC1 represented 44.25% of the variability and allowed to cluster samples according to the season (summer on the left, spring on the right) and independently to the sampling place (upstream (UP) or downstream (DO) the WWTP effluent). B) PCoA of eukaryotic community structure based on a Bray Curtis dissimilarity matrix. PC1 represented 29.44% of the variability and allowed to approximately cluster samples according to the season (spring on the left, summer on the right) and independently to the sampling place (upstream or downstream the WWTP effluent).
(▲) March-UP
(▲) March-DO
(■) April-UP
(■) April-DO
(●) May-UP
(●) May-DO
(●) June-UP
(●) June-DO
(●) July-UP
(●) July-DO
(●) August-UP
(●) August-DO

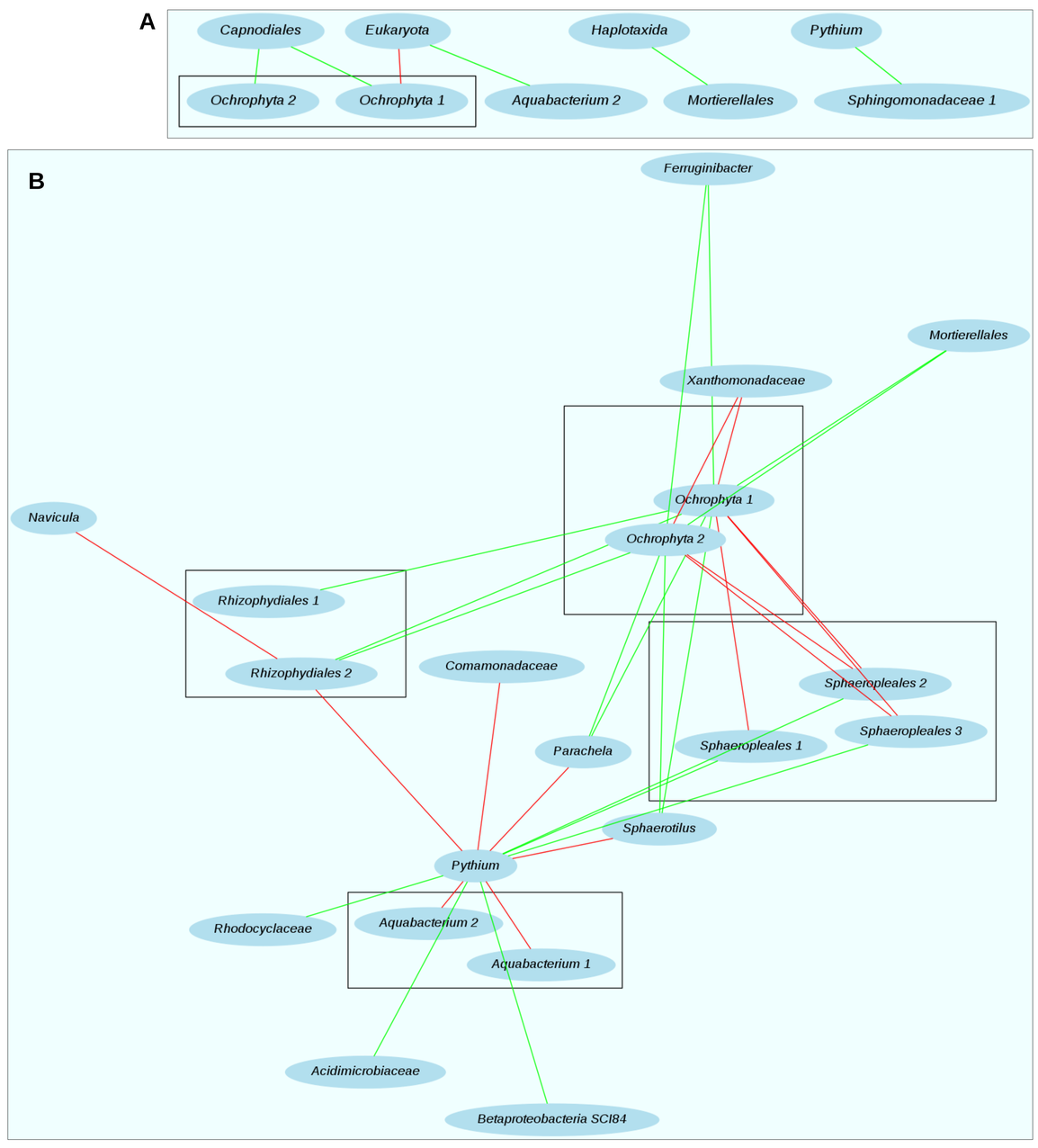

Figure 4: Potential variations in microbial interaction network. Spearman correlation coefficients were calculated between major 16S and 18S OTUs from upstream or downstream the WWTP effluent. Associated p-values were corrected using the Benjamini-Hochberg procedure (pFDR). A) Among the significant (pFDR < 0.05) positive (red lines) and negative (green lines) correlations, those present upstream and not downstream the WWTP effluent (pFDR > 0.5, to be sure they are really absent downstream) were represented. B) Among the significant (pFDR < 0.05) positive (red lines) and negative (green lines) correlations, those present downstream and not upstream the WWTP effluent (pFDR > 0.5, to be sure they are really absent upstream) were represented.

Conclusion

The taxonomic profile of microbial communities and the measure of the alpha diversity did not highlight significant differences between samples from upstream or downstream the WWTP effluent, suggesting a weak impact of this effluent containing pharmaceuticals molecules. Moreover, multivariate analyses show that the clustering of samples seemed more associated with seasons than with the sampling place. However, some significant correlations between the evolutions of microorganisms RA observed upstream the WWTP effluent were not observed downstream anymore. In the same way, there were several new significant correlations between the evolutions of microorganisms RA observed downstream the WWTP effluent which were not observed upstream. These significant correlations could represent interspecific relationships. Taken together, these results show that the WWTP effluent could perturb some interspecific relationships and could induce an adaptative response of some microorganisms. Further analyses will be necessary to evaluate the impact of these potential perturbations on ecosystems.