APPLICATION OF CYANOBACTERIA OLIGONUCLEOTIDE PROBES DESIGNED FOR IDENTIFYING BACTERIAL CELLS IN SURFACE WATER

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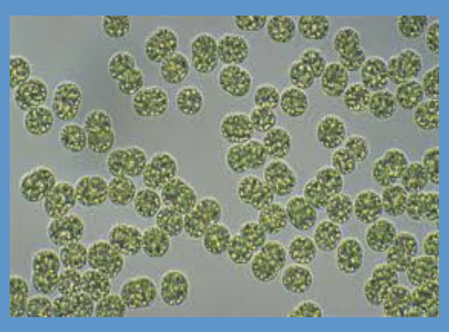
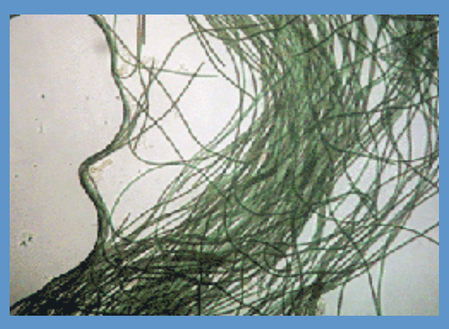
Cyanobacteria colonize different environments and blooms can occur both in contaminated and non-contaminated water bodies (freshwater, brackish and marine areas). In some cases, they can produce toxins and this phenomenon can produce a negative impact on ecosystem and human health. Among the main classes of cyanotoxins, microcystins are frequently found in surface water (Lucentini *et al*., 2011). *Microcystis aeruginosa* and *Planktothrix agardhii* are the most common species which can produce these toxins. The possibility to detect these species in aquatic environment using fast molecular based methods is a crucial point to assess the potential risk of their occurrence in natural and artificial water reservoirs (Mbedi *et al*., 2005).

Study Objectives:

- design, develop and validate oligonucleotide probes for Fluorescence *In Situ* Hybridization (FISH) analysis to detect *Microcystis aeruginosa* and *Planktothrix agardhii*.
- tested and validated with pure cultures and then field tested on natural water samples.





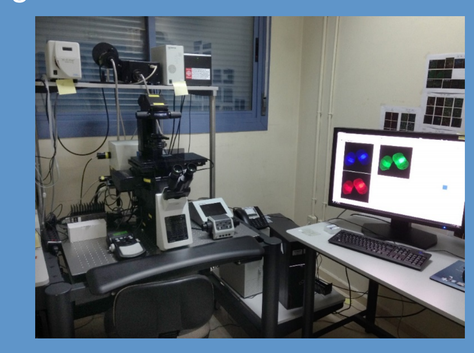
FISH probes

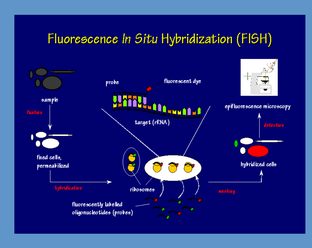
- designed using the ARB software (http://www.arb-home.de)
- *Microcystis aeruginosa:* genus (GNMICSO5) and species (MicAerD03)
- *Planktothrix agardhii;* genus (GNPlankS02) and species (PkAgD03) level probe
- Genus probes were labelled with Cy3 and the species ones with FITC.

Trials to reduce chlorophyll autofluoresecence (Medlin *et al.,* 2017)

- saline ethanol 1 hour or overnight
- +/- Dimethylformamide (50%).

➜ ➜ optimal results 1 hour saline ethanol + 1 50% Dimethylformamide

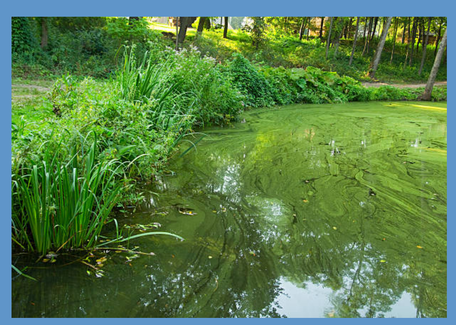




Probes applied to water samples collected from

- four different sampling points (1, 2, 3, 4) on Tiber River
- a volcanic lake (5) both located in Lazio region (central Italy).

Sampling Sites

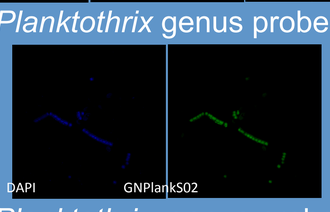


Planktothrix agardii

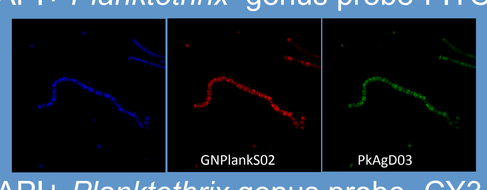
The GNPlankS02 genus probe was tested both in Cy3 and FITC and the species probe PkAgD03 was tested in FITC on a pure culture of *P. agardhii.* Images from a Confocal Laser Microscope.

Control

DAPI + *Planktothrix* genus probe- CY3



DAPI+ *Planktothrix* genus probe-FITC

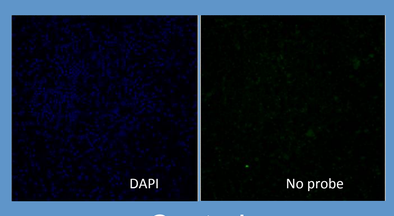


DAPI+ *Planktothrix* genus probe- CY3 + *Planktothrix agardii* species probe-FITC

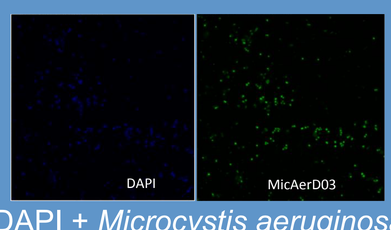
Microcystis aeruginosa

The GNMICSO5 and MicAerD03 probes were tested on a pure culture of *M. aeruginosa*.

- positive signal with the species MicAerD03 probe
- genus probe GNMICSO5 did not show an unequivocal signal.



Control



DAPI + *Microcystis aeruginosa* species probe-FITC

Results

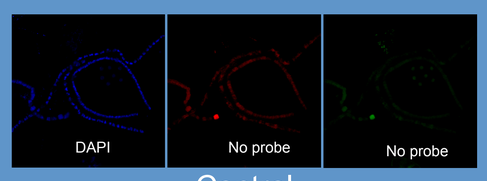
lake water samples

- positive signal to genus probe GNPlankS02.

river water samples

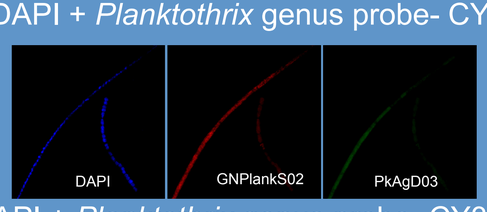
- positive signal to the MicAerD03 species probe in the contaminated sites 2, 3 and 4 low abundance.

- highest percentage of positive cells (8%) in the agriculture area(2) in Autumn sampling.

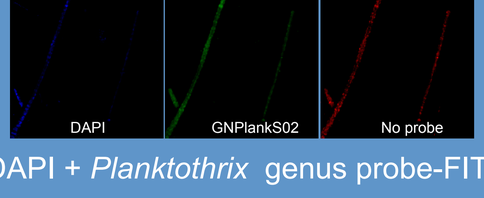


Control

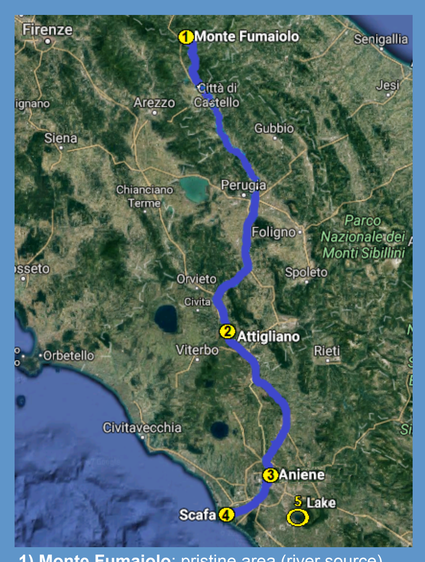
DAPI + *Planktothrix* genus probe- CY3



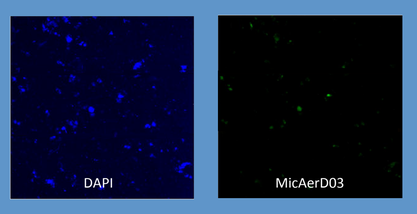
DAPI + *Planktothrix* genus probe- CY3 + *Planktothrix agardhii* species probe-FITC



DAPI + *Planktothrix* genus probe-FITC



1) Monte Fumaiolo: pristine area (river source)
2) Attigliano: agriculture area
3) Aniene: industrial contamination
4) Scafa: anthropogenic contamination
5) Volcanic lake: anthropogenic contamination



DAPI + *Microcystis* species probe-FITC
Autumn - Sampling point 2 (Attigliano)

The FISH probes were designed in the framework of the Marie Curie Actions - Industry-Academia Partnerships and Pathways **MicroCoKit** project N. 324518: *Microbial Community-based sequencing analysis linked to anthropogenic pressures: MicroCoKit to address the water quality.* MicroCokit is a close collaboration of academic groups and leading private enterprise to foster the transfer of knowledge among the partners with the final goal to bring to the market faster, sensitive and robust tools as bioindicators of water quality www.microcokit.eu.

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Mbedi S.,, Welker M, Fastner J, Wiedner C, 2005. *Variability of the microcystin synthetase gene cluster in the genus Planktothrix (Oscillatoriales, Cyanobacteria)*. *FEMS Microbiology Letters*, 245: 299–306.

Medlin LK, Guillebault D, Mengs G, Garbi C, Dejana L, Fajardo C, Martin M, 2017. *New molecular tools: application of the µAQUA phylochip and concomitant FISH probes to study freshwater pathogens from samples taken along the Tiber River, Italy.* In: Transactions on Ecology and the Environment, River Basin Management IX, vol. 221, WIT Press, in press.

Acknowledgements

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