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OLIGONUCLEOTIDE PROBES FOR FLUORESCENCE IN SITU IDENTIFICATION OF CYANOBACTERIAL CELLS IN SURFACE WATERS

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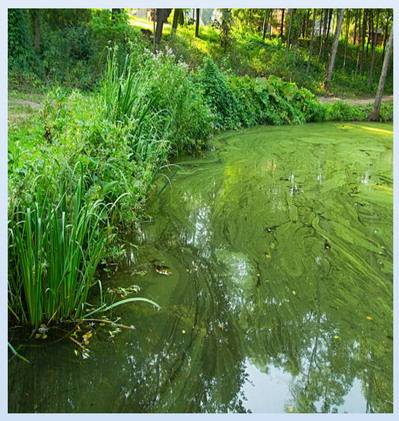
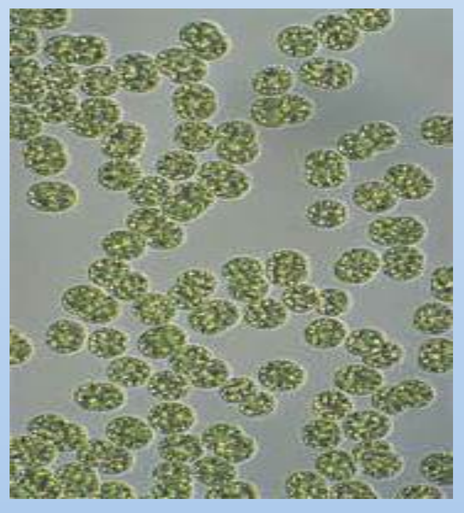
Harmful cyanobacterial blooms have been increasing in freshwater ecosystems in recent decades. In some cases, they can produce toxins which can have a negative impact on ecosystem and human health. Microcystins are the most frequently cyanotoxins found in surface water (Lucentini and Ottaviani, 2011). *Microcystis aeruginosa* is one of main toxic species. The possibility to detect promptly it in aquatic environment is a crucial point to assess the potential risk of its occurrence in natural and artificial water reservoirs (Mbedi et al, 2005). In the present work, we report the design, development and validation of two fluorescent oligonucleotide probes for detecting *M. aeruginosa* in natural freshwater samples using the Fluorescence In Situ Hybridization (FISH) method.



Main Objectives of the study:

- to design oligonucleotide probes for Fluorescence *In Situ* Hybridization (FISH) analysis to detect *Microcystis aeruginosa* in natural water samples
- to test and validate the probes designed with pure cultures
- to apply these probes to natural river water samples by FISH and CARD-FISH techniques



Oligonucleotide probes:

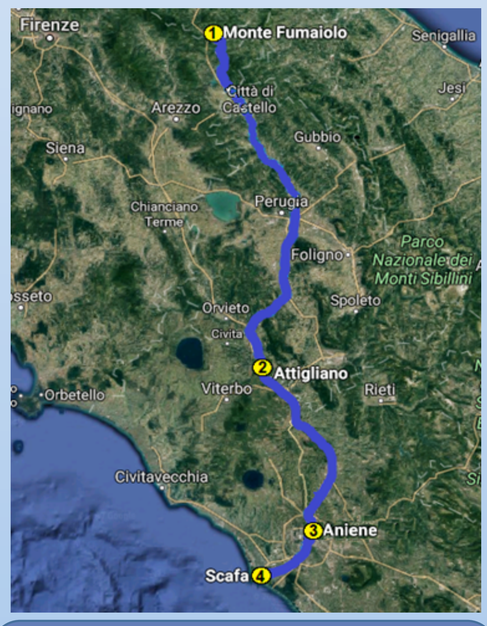
- designed using the ARB software: http://www.arb-home.de
- *Microcystis aeruginosa:* genus (GNMICSO5) and species (MicAerD03)
- Probes were labelled at the 5' end with FAM for FISH
- Probes conjugated at the 5' end with horse-radish peroxidase (HRP-MicAerD03) for CARD-FISH

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

- saline ethanol 1 hour or overnight
- +/- Dimethylformamide

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

Probes were then applied to river water samples collected from four different sampling points (1, 2, 3, 4) of the River Tiber in two different seasons (Autumn and Spring).



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

Application of the MicAerD03 probe to pure cultures of *Microcystis aeruginosa* by FISH

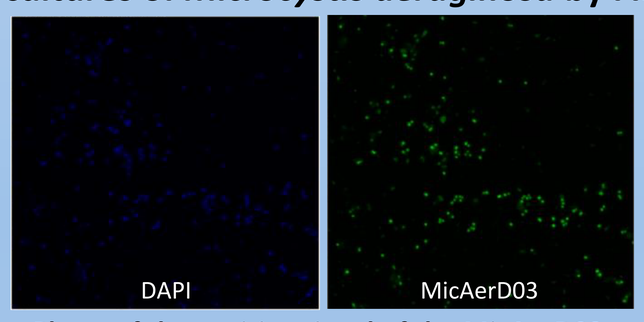


Photo of the positive signal of the MicAerD03 probe (green image) from a pure culture of *Microcystis aeruginosa*

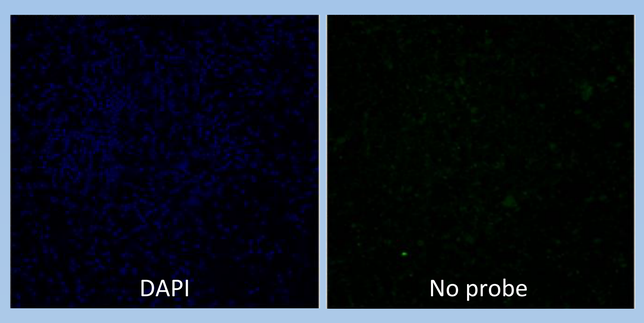


Photo of Control without probe

Application of the HRP-MicAerD03 probe to pure cultures of *Microcystis aeruginosa* by CARD-FISH

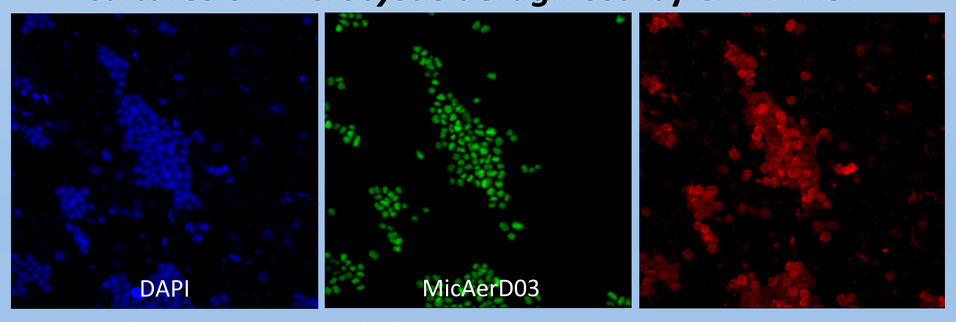


Photo of the positive signal of the HRP-MicAerD03 probe (green image) from a pure culture of *Microcystis aeruginosa*

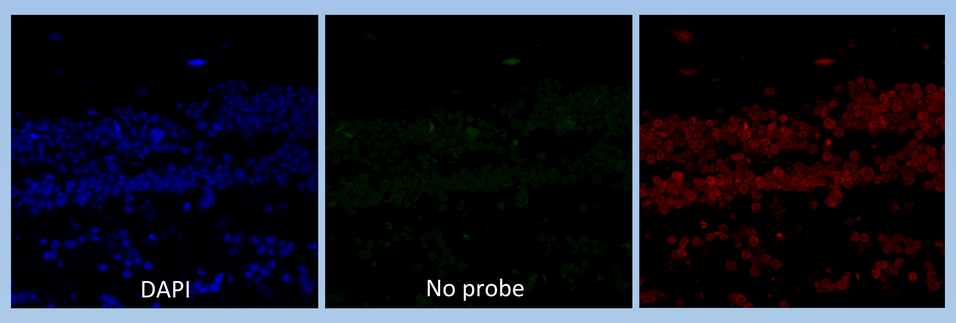
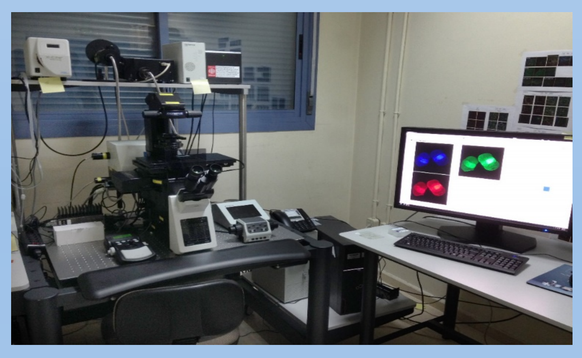


Photo of Control without probe



The positive signal of probe applications was detected under a Confocal Laser Microscope LEICA SP-2 AOBS

Application of the MicAerD03 probe to water samples from the River Tiber by FISH

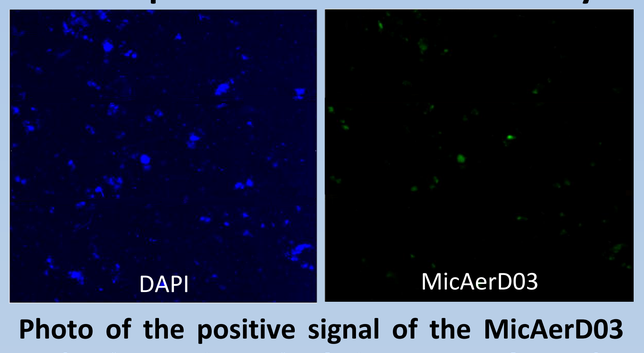


Photo of the positive signal of the MicAerD03 probe (green image) of river water from the sampling point 2 (Attigliano)

Application of the HRP-MicAerD03 probe to water samples from the River Tiber by CARD-FISH

Photo of the positive signal of the HRP-MicAerD03 probe (green image) of river water from the sampling point 1 (Monte Fumaiolo)

Concluding Remarks

- The FISH MicAerD03 probe was applied successfully both in the pure cultures of *M. aeruginosa* and in river water samples.
- *M. aeruginosa* was found, although in low abundance, in site 1, 2 and 3 of the river Tiber. The highest percentage of positive cells (8%) was found in the sampling point 2 in the Autumn sampling
- The species occurrence seems to be ubiquitous and its presence independent from the contaminant presence.
- *M. aeruginosa* was not found in the sampling point 4 (Scafa) corresponding to the river mouth, presumably due to the high NaCl concentration.
- The genus probe GNMICSO5 did not show an unequivocal signal and it was never used.

REFERENCES

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Acknowledgements

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