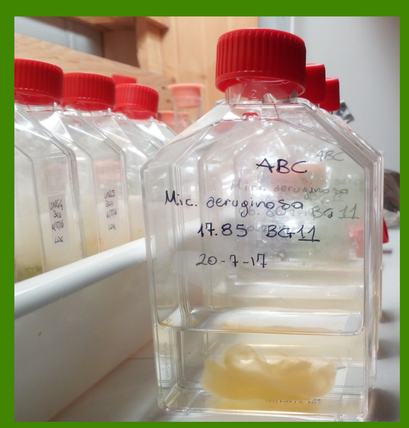
A NEW FLUORESCENT OLIGONUCLEOTIDE PROBE FOR IN SITU IDENTIFICATION OF MICROCYSTIS AERUGINOSA IN FRESHWATER

A. Barra Caracciolo[1], L. Dejana[1], C. Fajardo[2], P. Grenni[1], M. Martin[2], G. Mengs[3], S. Sánchez-Fortún[2], T. Lettieri[3], M.L. Saccà[1], L. Medlin[4]

[1] Water Research Institute-National Research Council (IRSA-CNR), Area della Ricerca RM1, Monterotondo, Rome, Italy
[2]Facultad de Veterinaria, Complutense University, Madrid, Spain. [3] Natural Biotec S.L., Parque Científico de Madrid, Madrid, Spain
[3] European Commission, Joint Research Centre (JRC), Directorate D-Sustainable Resources, Water and Marine Resources, Ispra (VA) – Italy
[4] Marine Biological Association of the UK, Plymouth UK

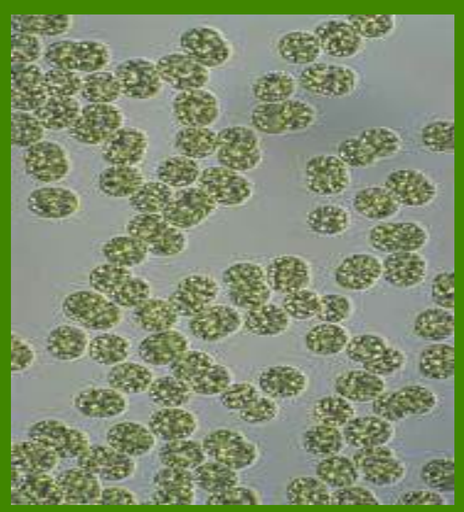
*Corresponding uthor: barracaracciolo@irsa.cnr.it

Cyanobacteria colonize different environments and blooms can occur both in contaminated and non-contaminated water bodies (freshwater, brackish and marine areas). Within 150 genera of cyanobacteria known, more than 40 species are able to produce toxins. The latter are natural compounds, which differ from both chemical and toxicological point of view and are responsible for both acute and chronic poisoning in animals and humans. Microcystins are among the most found in the environment.



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:

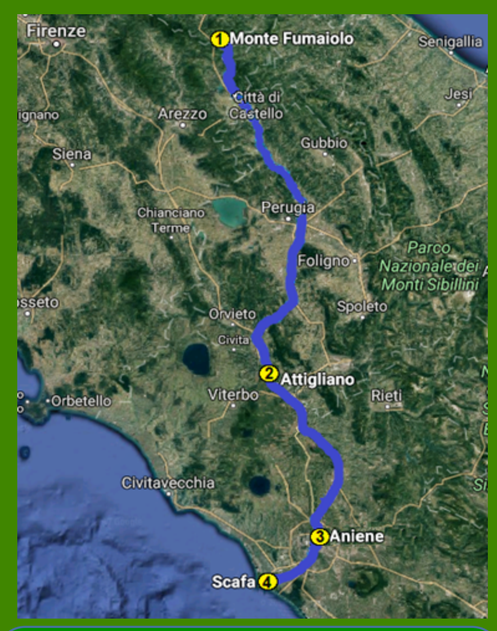
- designed using the ARB software: http://www.arb-home.de
- *Microcystis aeruginosa:* genus (GNMICSO5) and species (MicAerD03)
- Probes were labelled at the 5' end with FAM for FISH
- Probes conjugated at the 5' end with horse-radish peroxidase (HRP-MicAerD03) for CARD-FISH

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 FISH MicAerD03 probe to pure cultures of *M. aeruginosa*

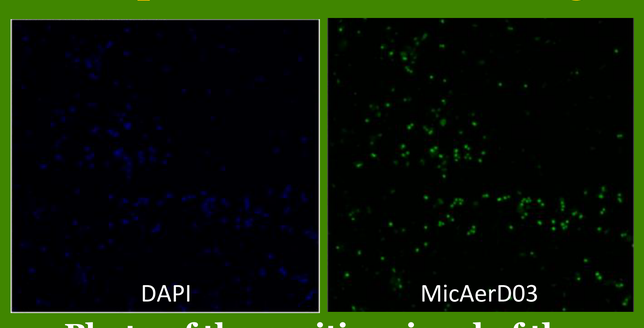


Photo of the positive signal of the MicAerD03 probe (green image) from a pure culture of *Microcystis aeruginosa*

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

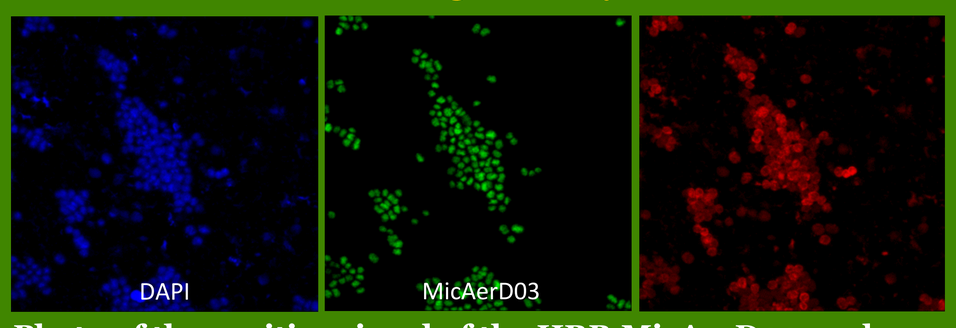


Photo of the positive signal of the HRP-MicAerD03 probe (green image) from a pure culture of *Microcystis aeruginosa*

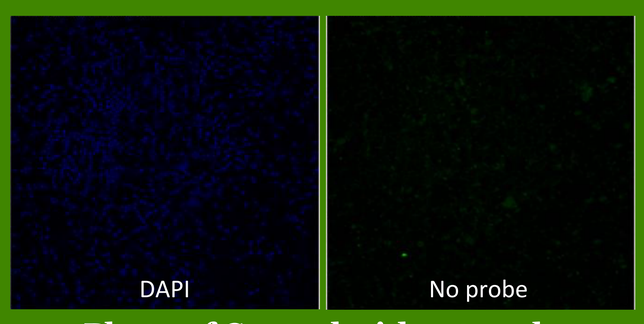
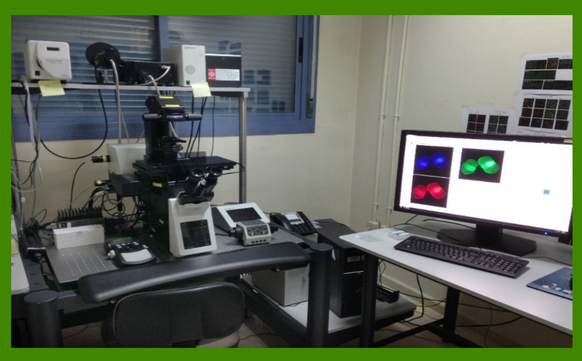


Photo of Control without probe

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 FISH MicAerD03 probe to water samples from the River Tiber

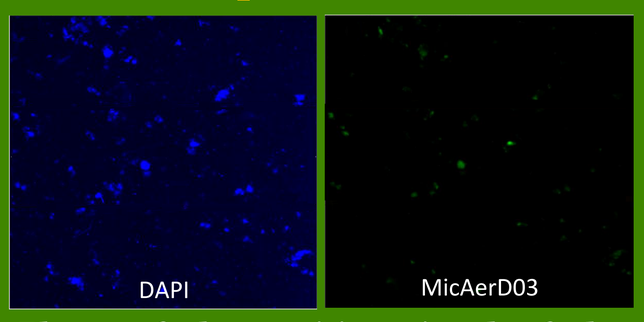


Photo of the positive signal of the MicAerD03 probe (green image) of river water from the sampling point 2 (Attigliano)

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

Lucentini L and Ottaviani M, 2011. *Cyanobacteria in water for human consumption: Guidelines for risk management*. National Group for cyanobacteria risk management in water for human consumption. Rapporti ISTISAN 11/35, 67 pp.

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: WIT Transactions on Ecology and the Environment, River Basin Management IX, WIT Press, vol. 221, 109-122.

Acknowledgements

Financial support was provided by the FP7-PEOPLE-2012-IAPP Industry-Academia Partnerships & Pathways - Marie Curie Actions Project MicroCokit N°324518: "*Microbial Community-based sequencing analysis linked to anthropogenic pressures: MicroCoKit to address the water quality*".
A thanks to Giulia Borlasco from EMBL (Italy) for her support in the image analysis.