INTRIGUING DIVERSITY AMONG DIAZOTROPHIC PICOPLANCTON ALONG A MEDITERRANEAN TRANSECT : A DOMINANCE OF RHIZOBIA

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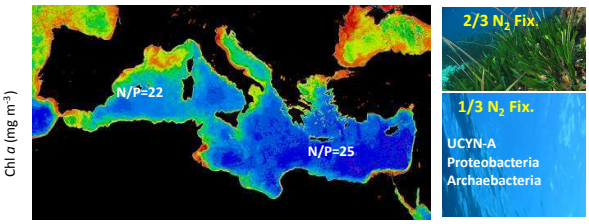
INTRODUCTION: Why are we studying diazotrophs in the Mediterranean Sea?

It is well known that the Mediterranean Sea has **unusual N/P** ratios compared to Redfield (N/P = 16), as riverine phosphate inputs are very low. Presence of nitrogen can be explained by i) low denitrification rates, ii) external nitrogen input by aerosols from urban and Saharan dust (Krom et al. 2010), and iii) significant diazotrophic activity (Béthoux and Copin-Montégut, 1986).



Trichodesmium, a well-known filamentous diazotrophic cyanobacteria, is significantly limited by low phosphate concentration and bloom of this species have been rarely reported in the Mediterranean Sea. As such, picoplanktonic (< 3 µm) microorganisms have been suggested as major diazotrophs (Béthoux and Copin-Montégut, 1986).

Béthoux and Copin-Montégut estimated that 2/3 of diazotrophic activity was due to microorganisms associated with marine sea-grass (Capone 1983), and 1/3 by planctonic microorganisms. 20 years later coastal investigations demonstrated the presence of these diazotrophs: UCYN-A (unicellular diazotrophic cyanobacteria from group A), Proteobacteria and Archaea on the South-East , as well as picoplanktonic and nanoplanktonic UCYN on the North-West (Man-Aharanovich et al. 2007 and Le Moal and Biegala 2009).

However planctonic diazotrophs biodiversity all the way through the Mediterranean Sea remained unknown.

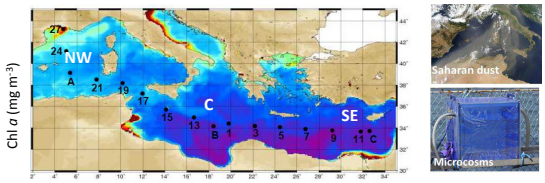
AIMS

► **Investigation of picoplanctonic diazotrophs biodiversity in the Mediterranean Sea**

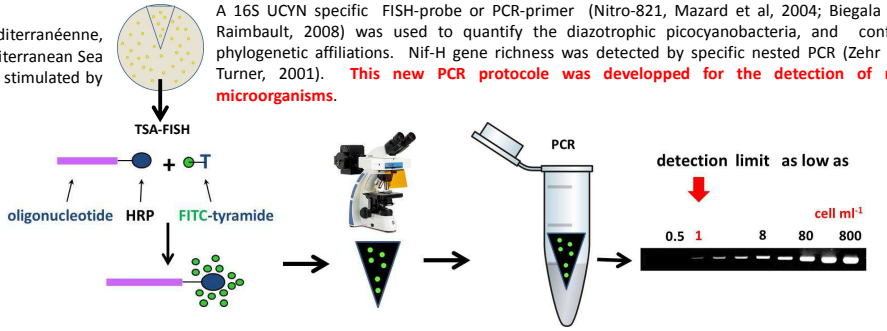
► **Development of new PCR approach to target rare populations of microorganisms**

MATERIALS AND METHODS

During the BOUM cruise (Biogéochimie de l'Oligotrophie à l'Ultra-Oligotrophie Méditerranéenne, Moutin et al. 2011) offshore samples were collected by filtration through the Mediterranean Sea for diazotrophs biodiversity investigations. At station A, B and C diazotrophs were stimulated by Saharan dust additions to microcosms.



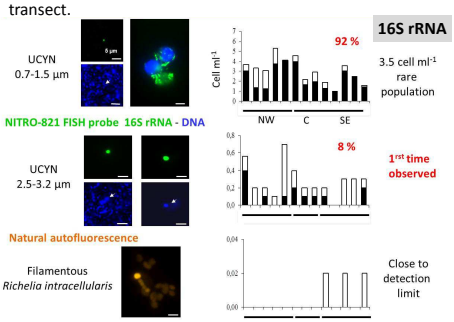
A 16S UCYN specific FISH-probe or PCR-primer (Nitro-821, Mazard et al, 2004; Biegala and Raimbault, 2008) was used to quantify the diazotrophic picocyanobacteria, and confirm phylogenetic affiliations. Nif-H gene richness was detected by specific nested PCR (Zehr and Turner, 2001). **This new PCR protocole was developped for the detection of rare microorganisms**.

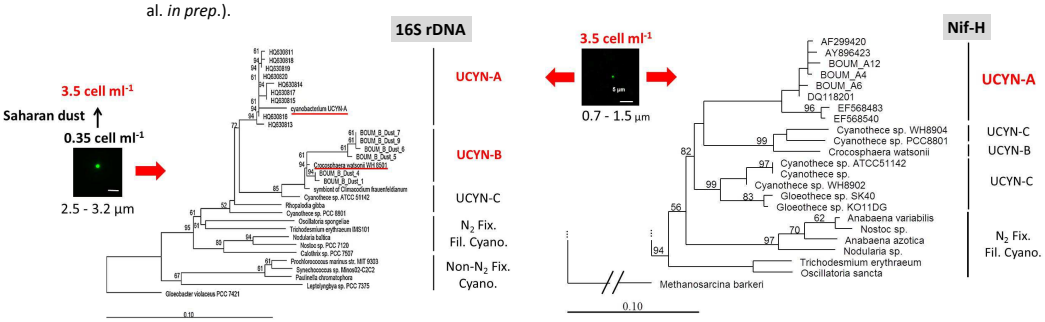


RESULTS ON DIAZOTROPHIC CYANOBACTERIA

Similar as previous coastal studies, offshore diazotrophic cyanobacterial community was dominated by picoplancton. Filamentous diazotrophs were close or below detection limit, only few *Richelia* were detected. Surprisingly among picoplanktonic population a larger cell type was discovered all through the transect.

Affiliation of small Nitro-821 targeted cells to UCYN-A was confirmed by 16S phylogeny using the same oligonucleotide sequence as the one used for **F**luorescent **I**n **S**itu **H**ybridization. Presence of **UCYN-A** was also confirmed by nif-H phylogeny. Saharan dust stimulated 10 times the larger picoplanctonic UCYN population, releasing the PCR detection limit and confirmed for the first time the presence of *Crocosphaera watsonii* (**UCYN-B**) all through the Mediterranean Sea (Hamza et al. *in prep.*).

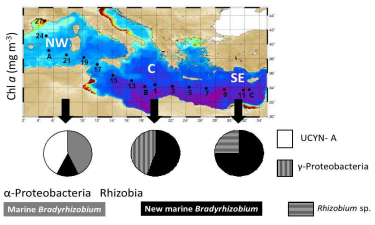


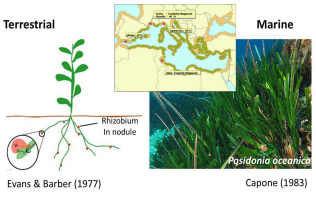


RESULTS ON DIAZOTROPHIC RHIZOBIAL

Rizobial nif-H sequences were dominating the Mediterranean Sea. Apart from UCYN-A and γ-Proteobacteria, all the other sequences were α-Rhizobial-Proteobacteria. Among them known marine Rhizobial were detected, as well as a new group of *Bradyrhizobium* spread all through the Mediterranean Sea.

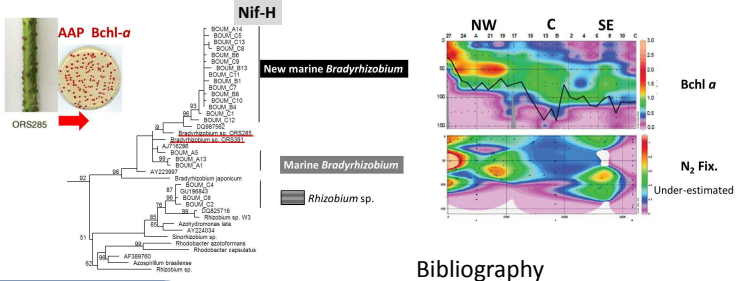
As rhizobial are efficient nitrogen fixers with higer plants such as terretrial crop and *Posidonia oceanica,* a Mediterranean seagrass, it is tempting to speculate that the three groups of known Rhizobia are free living stages of seagrass symbionts deprived from autonomous diazotrophic activity.





The new marine group of ***Bradyrhizobium*** have its closest relative among freshwater strains which can fix nitrogen extra-plantae, an unusual metabolic activity among Rhizobial (Giraud and Fleischman, 2004). These **A**erobic bacteria have bacteriochlorophyl-a and realise **A**noxygenic **P**hotosynthesis (**AAP**).

AAP marine nitrogen fixers have not been discovered yet and lateral nif-H gene transfer is well spread, thus diazotrophic marine *Bradyrhizobium* remains to be demonstrated, although it is interesting to see the match between nitrogen fixation and bacteriochlorophyll-a distribution during the BOUM cruise (Bonnet et al., 2011; Lamy et al., 2011).



CONCLUSIONS

► Picoplanktonic UCYN-A and UCYN-B are rare populations of diazotrophic cyanobacteria and are well spread all through the Mediterranean Sea.

► Numerous nif-H sequences of *Bradyrhizobium* suspect the dominance in the Mediterranean Sea of a new marine free living picoplanktonic diazotroph with **A**erobic **A**noxygenic **P**hotosynthesis ability.

► Combination of FISH technique with PCR on entire cells collected by filtration can decrease detection limit as low as 1 cell.ml^{-1} and allow to target species richness of rare microorganism populations.

Bibliography

Béthoux and Copin-Montégut, 1986. Limnology and Oceanography, 31 : 1353-1358

Biegala and Raimbault, 2008. Aquatic Microbial Ecology, 51:45-53.

Bonnet et al., 2011. Biogeosciences, 8: 2257-2267.

Capone, 1983. Nitrogen in the marine environment, ed Carpenter EL and Capone DG, Academic,: 105-137.

Giraud et Fleischman, 2004. Photosynthetic Research, 82: 115-130.

Hamza et al., in prep. Nutrients and Saharan dust events control unicellular diazotrophic cyanobacterial development in oligotrophic Mediterranean Sea.

Krom et al., 2010. Progress in Oceanography, 85: 236-244

Lamy et al., 2011. Biogeosciences, 8 : 973-985.

Le Moal and Biegala, 2009. Limnology and Oceanography, 54:845-855.

Man-Aharanovich et al., 2007. Environmental Microbiology, 9:2354-2363.

Mazard et al., 2004. Applied Environmental Microbiology, 70:7355-7364.

Moutin et al., 2011. Biogeosciences, 9:3817-3825.

Zehr and Turner, 2001. Methods in Microbiology, 30:271-286.