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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).

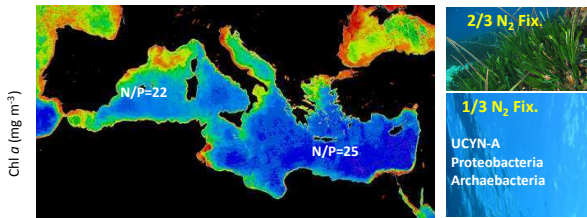
Trichodesmium, a well-known filamentous diazotrophic cyanobacteria, is significantly limited by low phosphate concentration and bloom of this species have been rarely reported in the Mediterranean Sea. As such, picoplanktonic (< 3 μm) microorganisms have been suggested as major diazotrophs (Béthoux and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

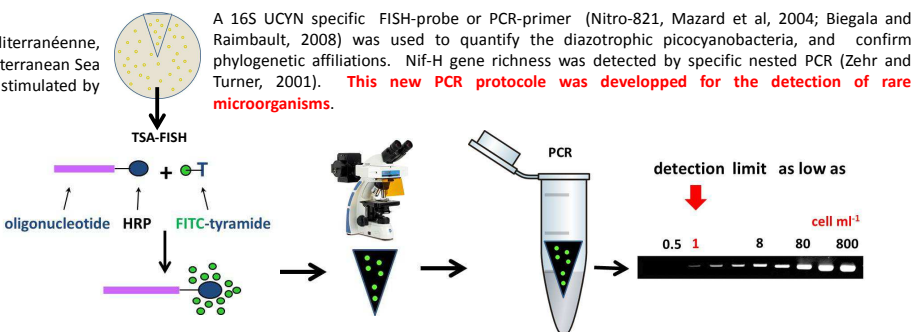
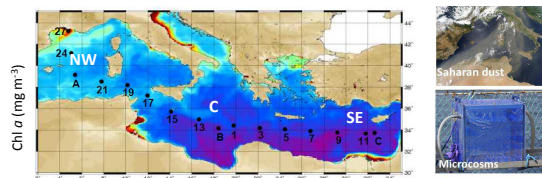
AIMS

- Investigation of picoplanktonic diazotrophs biodiversity in the Mediterranean Sea
- Development of new PCR approach to target rare populations of microorganisms



MATERIALS AND METHODS

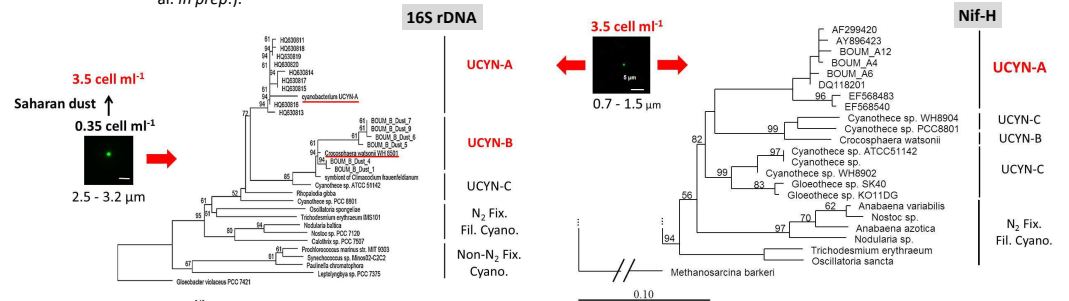
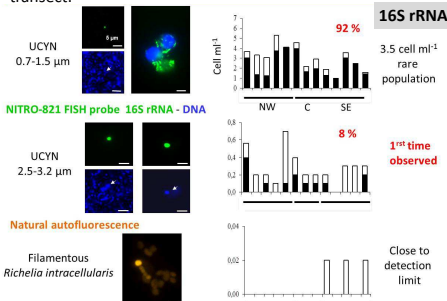
During the BOUM cruise (Biogéochimie de l'Oligotrophie à l'Ultra-Oligotrophie Méditerranéenne, Moutin et al. 2011) offshore samples were collected by filtration through the Mediterranean Sea for diazotrophs biodiversity investigations. At station A, B and C diazotrophs were stimulated by Saharan dust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



RESULTS ON DIAZOTROPHIC CYANOBACTERIA

Similar as previous coastal studies, offshore diazotrophic cyanobacterial community was dominated by picoplankton. 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 Fluorescent In Situ Hybridization. Presence of UCYN-A was also confirmed by nif-H phylogeny. Saharan dust stimulated 10 times the larger picoplanktonic UCYN population, releasing the PCR detection limit and confirmed for the first time the presence of *Crocospaera watsonii* (UCYN-B) all through the Mediterranean Sea (Hamza et al. in prep.).

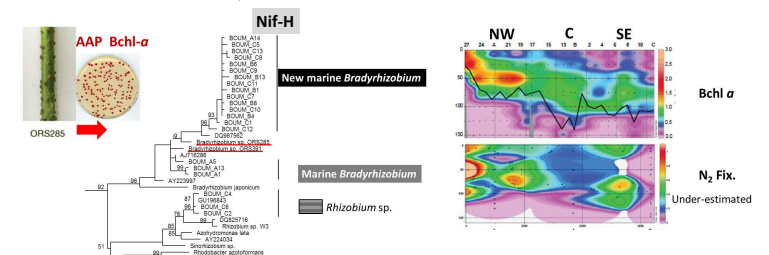
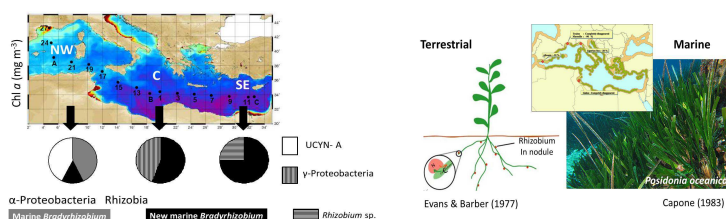


RESULTS ON DIAZOTROPHIC RHIZOBIAL

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.

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 Aerobic bacteria have bacteriochlorophyll-a and realise Anoxygenic Photosynthesis (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).

As rhizobial are efficient nitrogen fixers with higher plants such as terrestrial 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.



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 Aerobic Anoxygenic Photosynthesis 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.

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