Relationships between genetic diversity and niche-partitioning of *Synechococcus* populations in Mediterranean coastal lagoons, systems of transition between freshwater and marine ecosystems

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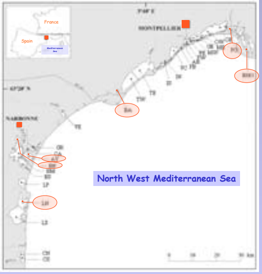
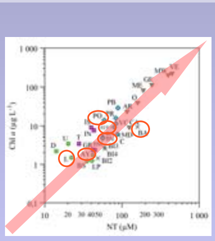
Coastal lagoons are morphologically and ecologically complex ecosystems, subject to constantly changing environmental conditions generally of much greater magnitude than it is recorded in the open sea. Due to their transitional nature, they usually display a number of specific features (ie. high nutrient concentrations, light spectral quality, salinity range) which are potentially influencing strategies of adaptation of photosynthetic microorganisms. The unicellular cyanobacteria *Synechococcus* is a major component of marine and freshwater picophytoplankton. Its genetic diversity is well documented for these ecosystems, but knowledge about biodiversity and distribution of this genus in transitional waters remains scarce. The phylogenetic analysis of a library of 240 *Synechococcus* environmental clones (16S rDNA sequences) isolated from different coastal lagoons reveals that these transitional waters are populated with diverse *Synechococcus* strains including members from freshwater lakes, brackish, coastal and marine (sub-clusters 5.1 and 5.2) waters. This phylogenetic analysis was combined with an analysis of population genetics in order to understand the relationships between patterns of community structure and environmental parameters.

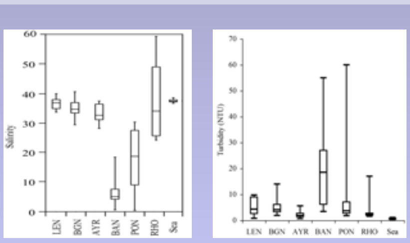
Environmental variability of the studied Mediterranean coastal lagoons



Lagoons	SALSES-LEUCATE LEN	BAGES-SIGEAN BGN	AYROLLE AYR	GRAND BAGNAS BAN	PONANT PON	RHONE DE SAINT ROMANS RHO
Area (ha)	5400	3700	1320	150	200	50
Mean depth	2.1-1.5	1,3	0,5	0.4		-
Max depth	3,7	2.85	-	-	5	-
Volume ($10^6.m^3$)	90	0.6	9	0,6		
Salinity	37.8	29.1	32	11.4	21.5	51.4
Direct opening on the sea	3	1	1	0	1	1-Human activity
Main brakishwater provider	-	-	-	Canal du Midi	-	-
Main freshwater provider	watershed	Canal de la Robine	Campignol lagoon	watershed	Vidourle river	watershed
Trophic status according Smith et al 1999	oligotrophic	mesotrophic	mesotrophic	eutrophic	hypertrophic	hypertrophic



Plot of median **Chla concentrations versus TN concentrations** in the six studied French Mediterranean lagoons from 1998-1999 annual cycles data for LEN, BGN, AYR, 2001-2002 for BAN, PON and 2003-2004 for RHO.



Distribution of **salinity** and **turbidity** in the six studied French Mediterranean lagoons from 1999-1998 annual cycles data for Sea, LEN, BGN, AYR, 2001-2002 for BAN, PON and 2003-2004 for RHO.

Mediterranean coastal lagoons comprise strong gradients of nutrients and salinity leading to various ecological niches.

Picophytoplankton abundance and composition

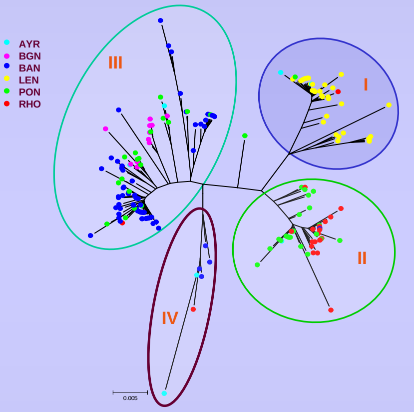
Picophytoplankton (≤ 3µm), determined by flow cytometry, is composed of phycoerythrin-rich cyanobacteria (PE-CYAN), phycocyanin-rich cyanobacteria (PC-CYAN) and picoeukaryotes (PEUK). Values have been obtained from samples collected in july 2005.

Lagoons	Picoplanktonic cells x 10^6/L	Chlorophyll *a* µg/L	% PE-CYAN1 High PUB/PEB	% PE-CYAN2 Low PUB/PEB	% PC-CYAN	% PEUK
LEN	169,99	1,99	8,7	90,6	0,0	0,6
BGN	9,43	6,72	0,0	5,6	43,8	50,6
AYR	7,90	1,14	3,5	0,0	14,4	82,2
PON	987,18	35,6	0,1	0,8	82,1	17,0
RHO	2622,00	11,1	0,0	0,5	99,1	0,4
BAN	5935,28	93,1	0,0	0,0	77,6	22,4

Lagoons	Nucleotide diversity (π)
AYR	0.031
BAN	0.013
BGN	0.023
LEN	0.009
PON	0.019
RHO	0.014

Comparison of the levels of nucleotide diversity within *Synechococcus* populations is a first step which may provide valuable information for appraising genetic structure infered from speciation and/or dispersal process.
Globally, the calculated degrees of polymorphism of *Synechococcus* populations from the lagoons are relatively low. The lowest degree of polymorphism is observed for the oligotrophic lagoon LEN which is the most oligotrophic ecosystem with an high PE-Cyan relative abundance. The highest nucleotide diversity is recorded in the mesotrophic lagoon AYR where picoeukaryotes are the major components of the picophytoplanctonic community. Both lagoons display a low salinity variability. The eutrophic (BAN) and hypertrophic (PON and RHO) with high PC-Cyan abundances present a low diversity of *Synechococcus* ribotypes. The mesotrophic BGN lagoon holds out an intermediate nucleotide diversity of its *Synechococcus* population, the picophytoplanctonic community is made up for half of PE-PC-Cyan and picoeukaryotes.

Comparative analysis of population genetic structure



Phylogenetic relationships among the environmental sequences from Salses-Leucate, Bages-Sigean, Ayrolle, Grand Bagnas, Ponant and Rhône de Saint Romans lagoons . The unrooted tree was constructed from pairwise Tamura and Nei distance by using the Neighbour-Joining method

1- **The phylogenetic analysis** of the clone library reveals **evidence for restricted distribution of *Synechococcus* ribotypes to specific geographical locations** both among and within lagoons:

Clade I gathers together all the ribotypes isolated from the oligotrophic LEN lagoon.
Clade II groups together ribotype detected in the most eutrophic lagoons PON (57%) and RHO (86%).
Clade III groups 93% of the ribotypes isolated from BAN, 39% of the ribotypes from PON.
6 ribotypes isolated from BAN, AYR and RHO also form a separating clade IV
AYR ribotypes are distributed in clade I, II and IV

2- **AMOVA analysis and Mantel tests:**

The main environmental parameters, i.e. salinity, trophic status (chlorophyll *a* concentrations), salinity variability which can be considered here as a parameter of exchange between land-lagoon-sea, discriminating the studied lagoons were tested. **AMOVA analysis and FST values show that the genetic variance is significantly explained by the structure of the populations** and is not significantly explained by their geographical area of isolation (among groups and among popualtion within groups). Mantel tests also do not show that the genetic diversity is linked to these environmental conditions.

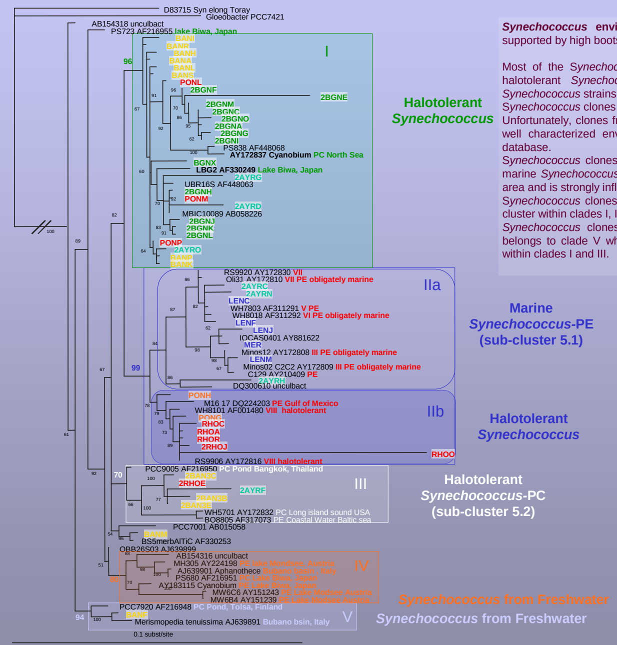
Case 1: AMOVA analysis with two groups of lagoons according "Salinity variability": BAN-PON-RHO and BGN-AYR-LEN

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	480.675	2.34053 Va	12.35
Among populations within groups	4	839.381	6.59853 Vb	34.82
Within populations	205	2052.846	10.01388 Vc	52.84
Total	210	3372.902	18.95295	

Fixation Indices
FSC*** : 0.39720
FST*** : 0.47165
FCT*** : 0.12349

***P<0.0001

Phylogenetic analysis

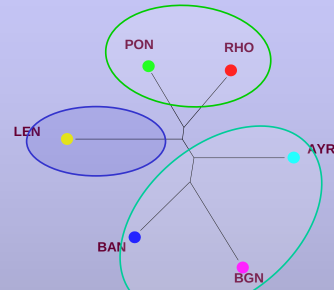


Maximum likelihood 16S rRNA tree showing the relationship of *Synechococcus* environnemtal sequences from the six studied Mediterranean lagoons Salses-Leucate (LEN),Bages-Sigean (BGN),Ayrolle (AYR), Grand Bagnas (BAN), Ponant (PON) and Rhône de Saint Romans (RHO) and cultured *Synechococcus* strains.
The tree was constructed with PAUP4.0b. Bootstrap supports were estimated by Phyml (1000 replications). The values above 50 % are indicated for each node. The branch leading to the outgroups (double bars) was shortened for graphical reasons.

***Synechococcus* environmental clones** fall into five distinct clades, which are well supported by high bootstrap values.

Most of the *Synechococcus* clones isolated in the studied transitional waters belong to halotolerant *Synechococcus* clades I, IIb and III which contain PC and PE-rich *Synechococcus* strains
Synechococcus clones isolated from Bages-Sigean lagoon belong to the halotolerant clade I.
Unfortunately, clones from clade I do not correspond to any closely related sequences from well characterized environmental samples or cultures published in the public GenBank database.
Synechococcus clones isolated from Salses-Leucate lagoon cluster only with the IIa clade marine *Synechococcus* (sub-cluster 5.1). This lagoon is slightly influenced by its catchment area and is strongly influenced by the marine environment.
Synechococcus clones isolated from Ayrolle, Ponant and Rhône de Saint Romans lagoons cluster within clades I, IIb and III which gather together halotolerant and marine strains.
Synechococcus clones from Grand Bagnas lagoon except from one clone (BANF) that belongs to clade V which represents *Synechococcus* from freshwater ecosystems, cluster within clades I and III.

Population tree



The population tree shows that PON and RHO, the most eutrophic lagoons cluster together. They are very close but no connection is observed between them. LEN, the marine lagoon, is isolated from the others. BGN, BAN and AYR cluster together which can be put in relation to the fact that they are connected with canal du Midi and canal de la Robine.

Conclusions

Our clone library of 16S rDNA partial sequences suggests that more *Synechococcus* clades exist than those which are currently in culture. The genetic diversity of PC-Cyan population is poorly documented and the genome sequence of the *Synechococcus* CC9311 strain will help to understand adaptation to a coastal envitonnement. Nevertheless, it will be interesting to isolate more cultured cyanobacterial clones in order to study their physiological properties. Cytometric data show also that some PE-Cyan are able to grow in eutrophic conditions.

The population tree shows that geographical isolation and connections via several canals might play a major role in the distribution of the *Synechococcus* ribotypes and there is an extensive dispersal of some closely related taxa, which are able to grow in very diverse conditions of nutrients availability and salinity and with specific chromatic adaptation capability. Multiple ecotypes might play a major role in the ecological success of *Synechococcus* in transitional waters.

Phylogenetic analysis with studied *Synechococcus* strains suggests a strong link between genetic diversity and the main forcing environmental factors describing these transitional ecosystems. This observation is in agreement with the « assumption of niches conservation hypothesis » (Wilson, 1994) which is based on the postulate that each species occupies a different ecological niche within which it is the best competitor for given resources or environmental factors.

However, our populations analysis shows that there is no significant association between distribution of *Synechococcus* ribotypes and ecological variables. The *Synechococcus* community can be considered, in that case, as an assembly of species determined by dispersion and not by the available niches. It is the « dispersal assembly perspective » (Hubbel, 2001) where the dissemination is fundamental and constitutes the principal spatial limitation for the colonization of favorable locations.

In conclusion, our results suggest that both hypothesizes described above (adaptation and dispersion) are not mutually exclusive and their respective importance remains to be establish, likewise the factors likely to vary it.