

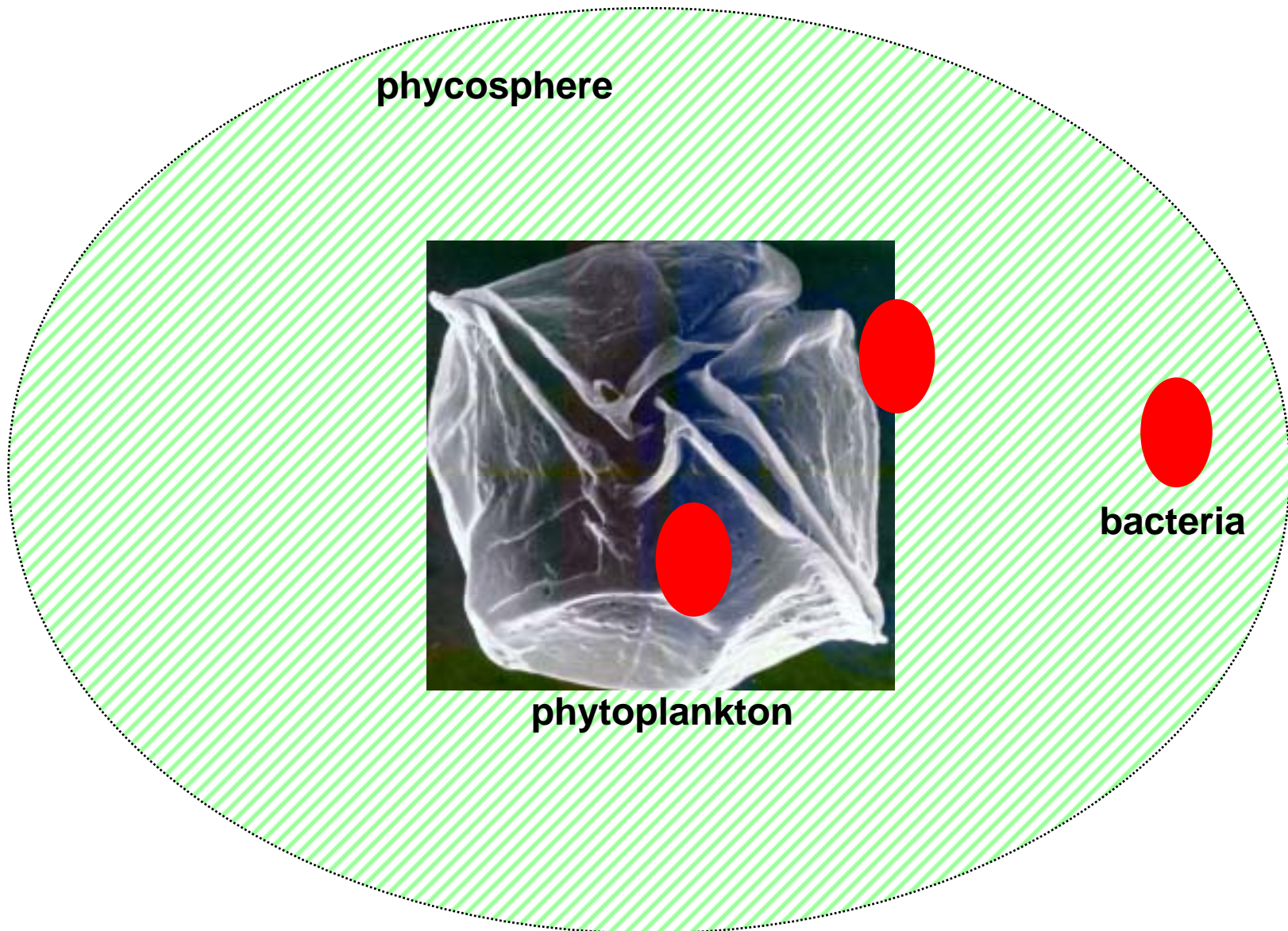
# **Identification of bacteria associated with dinoflagellates using TSA-FISH and confocal microscopy**

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G. Kennaway, E. Alverca, J.-F. Lennon, D. Vaultot, N. Simon**

J. Phycol. 38 (2002)

**(CNRS, Biological Station of Roscoff, France)**

# Spatial relationships dinoflagellates/bacteria



# Importance of studying dinoflagellates/bacteria relationships

Important members in  
marine pelagic ecosystems

Involved in  
Harmful Algal Blooms

Identification

+

Localization

**TSA-FISH**

**Confocal microscopy**



Specificity of



interactions

# Method: Fluorescent *in situ* hybridization

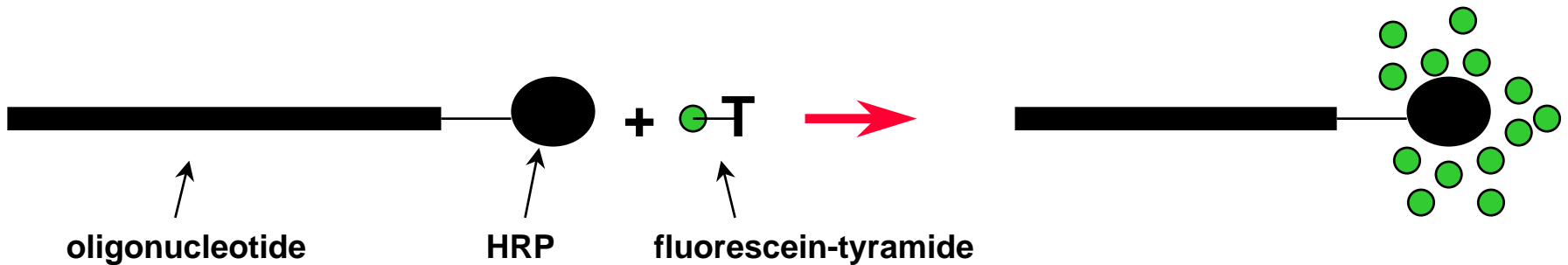
1- Fixation, dehydration, permeabilization

2- FISH:

- Monolabeled probes



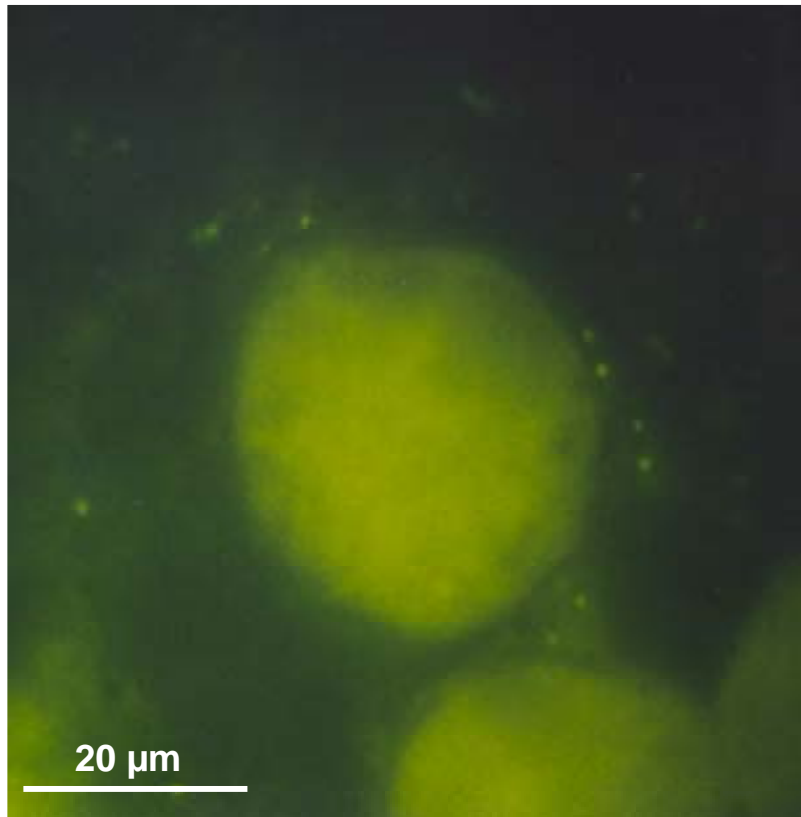
- Tyramide Signal amplification



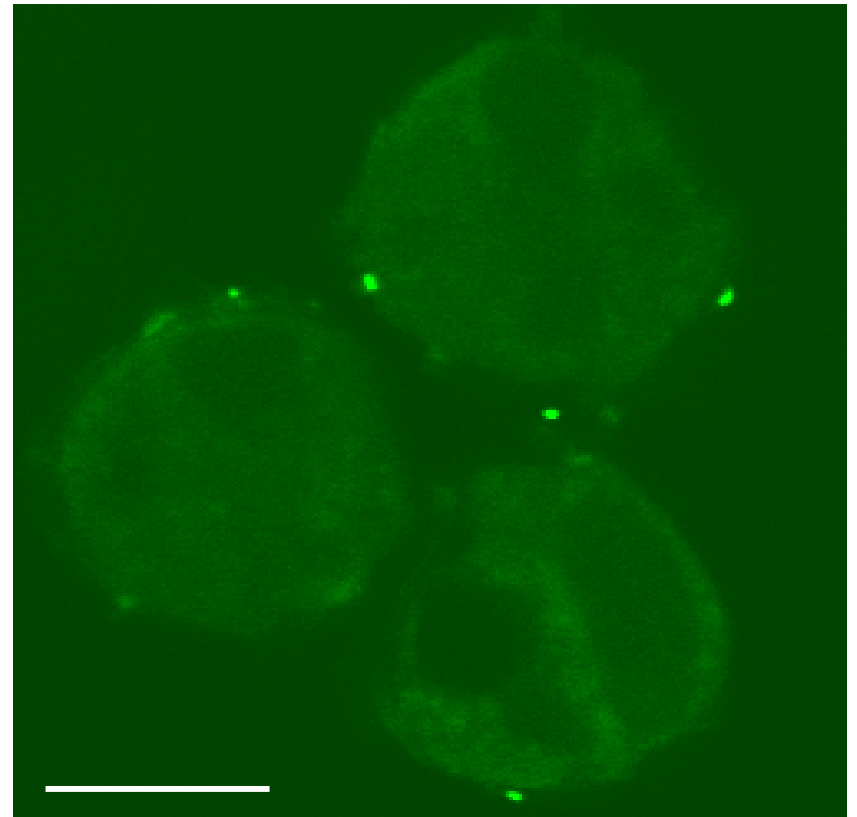
# Localization of associated bacteria

*Alexandrium tamarense* + bacteria (**Eubacteria-probe**)

Epifluorescence microscope (400 - 500 nm)

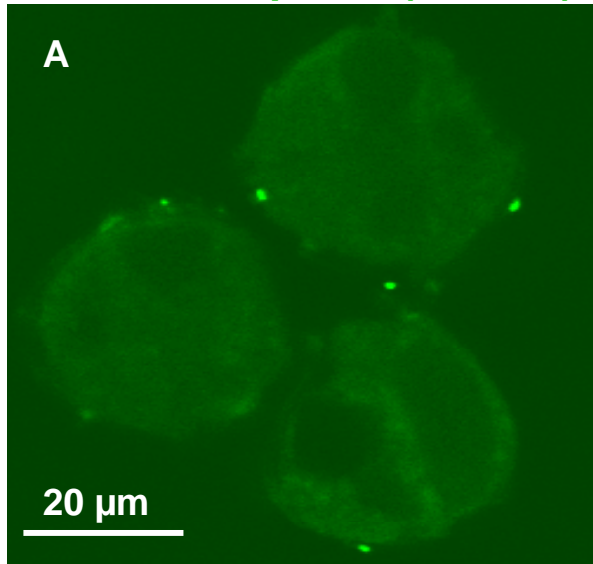


Confocal microscope (488 nm)

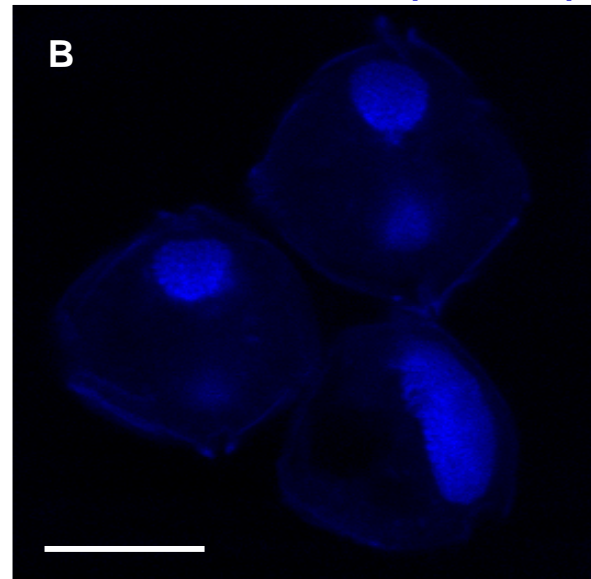


# Localization of attached bacteria

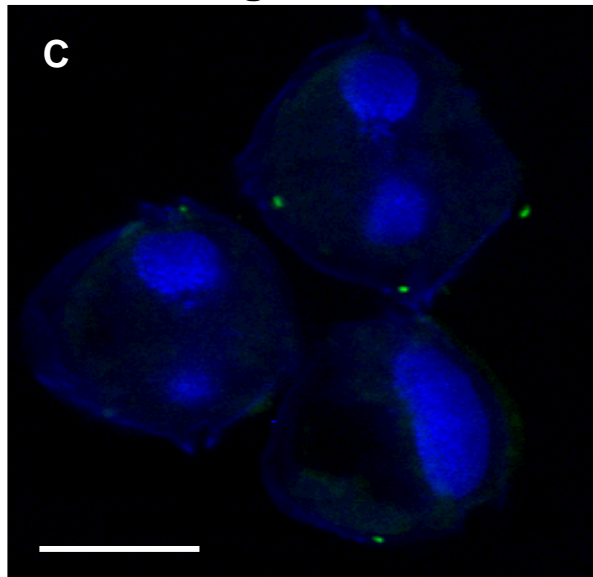
Eubacteria-probe (488 nm)



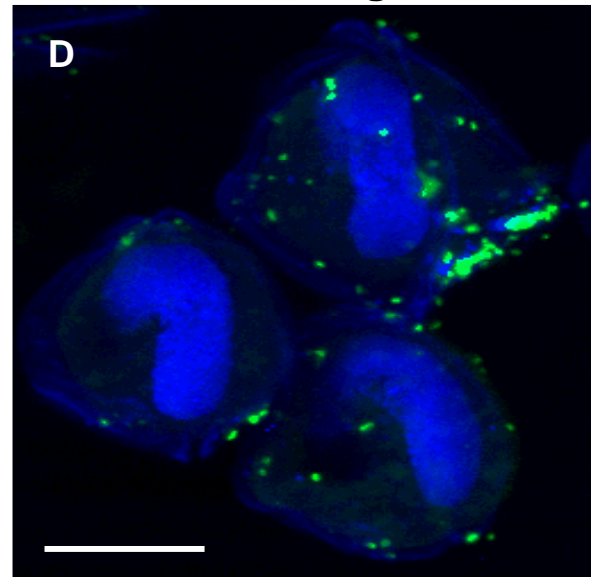
DAPI & calcofluor (380 nm)



Merge of A + B



Addition of merged slices

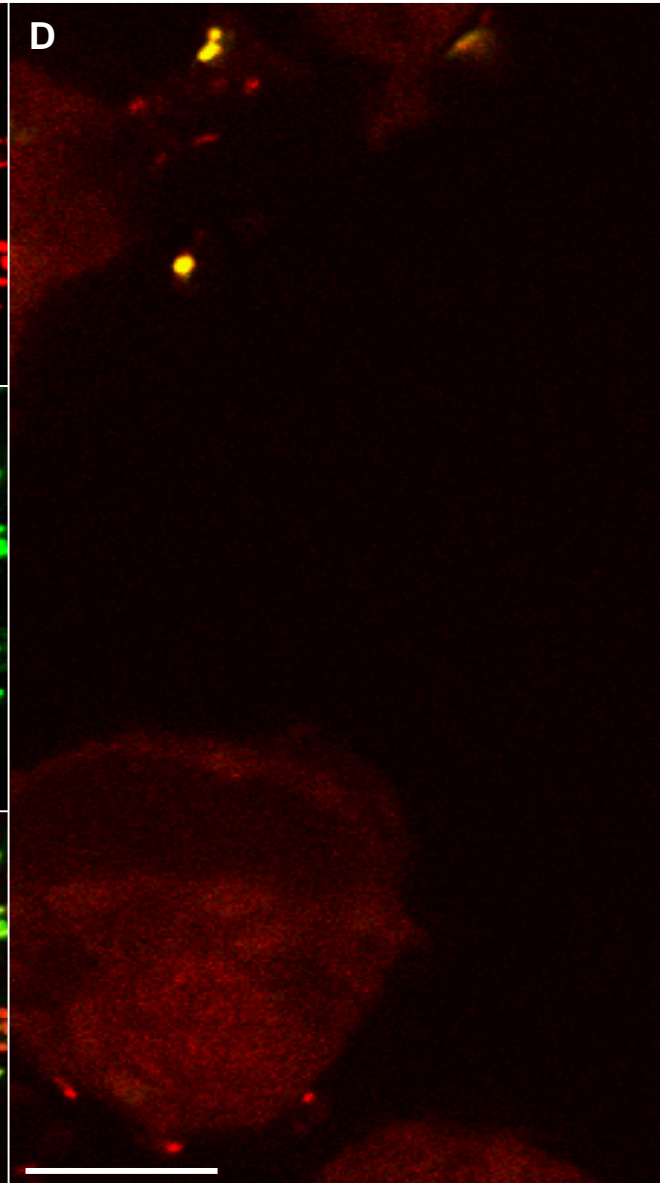
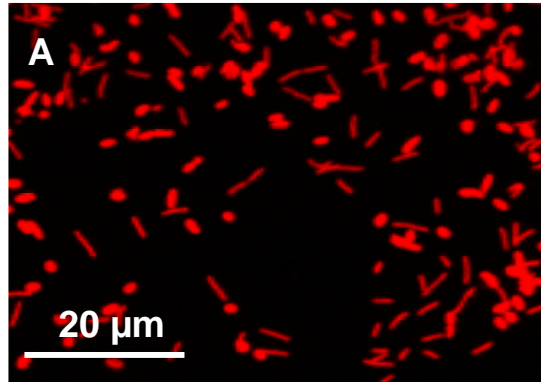


# Identification of attached bacteria: double hybridization

Free bacteria

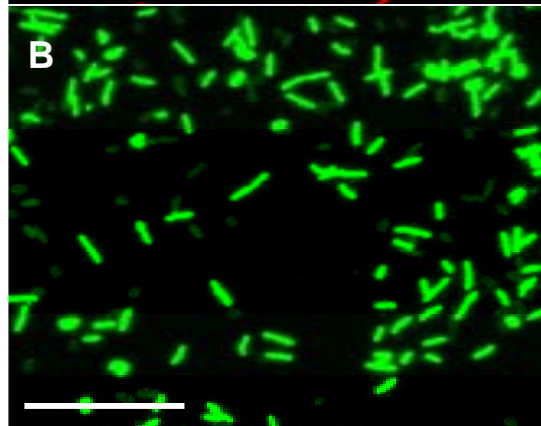
*A. tamarensis* + bacteria (Merged slices)

Eubacteria-probe  
(568 nm)



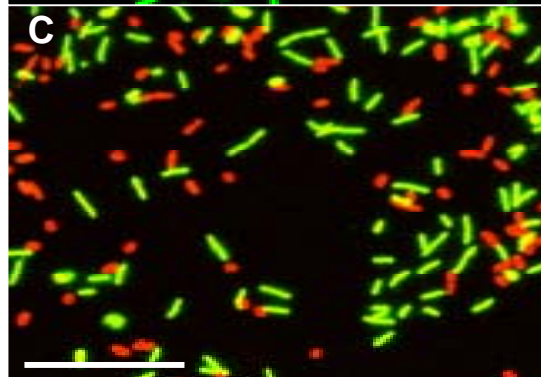
Eubacteria  
probe  
(568 nm)

Cytofaga-Flavobacter  
probe  
(488 nm)



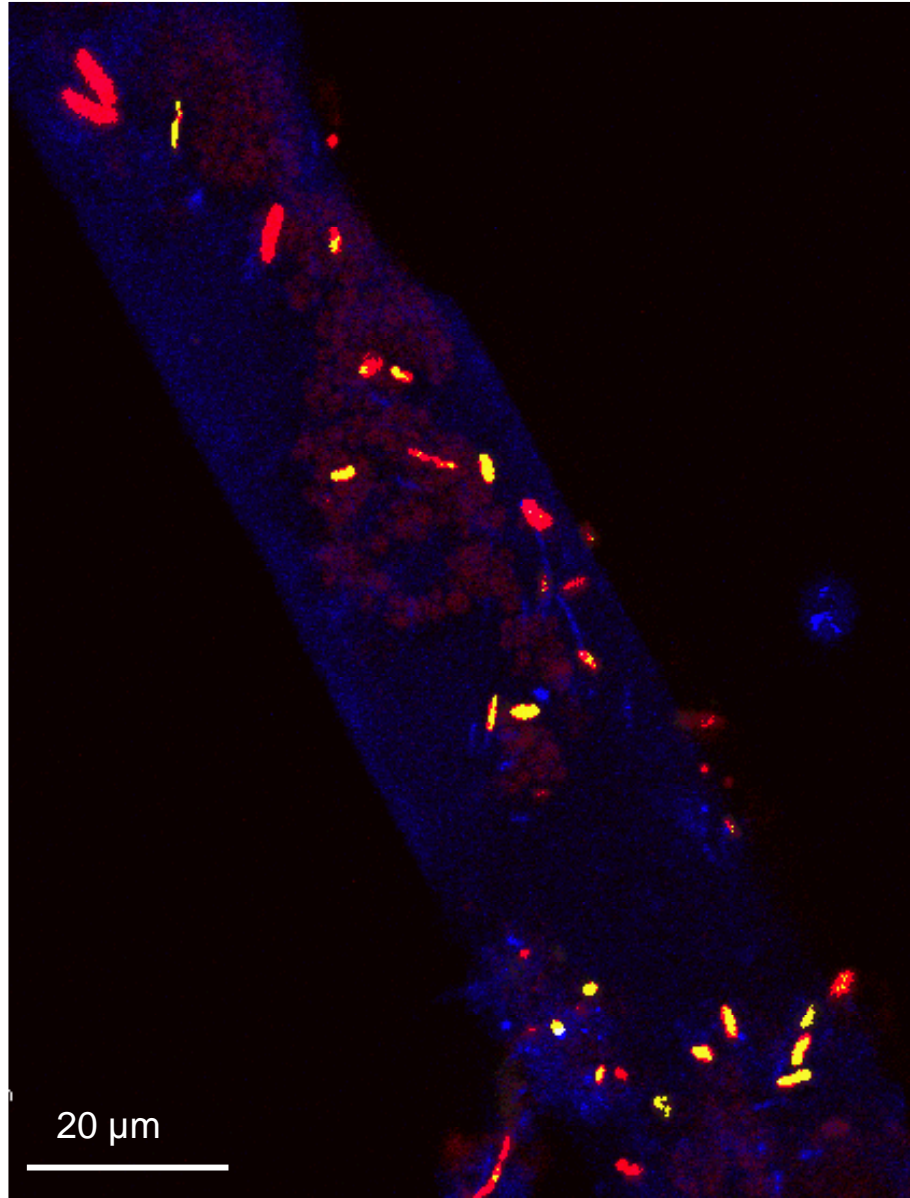
Roseobacte  
probe  
(488 nm)

Merge of A + B



# Identification of attached bacteria in mixed phytoplankton

Eubacteria-probe (568 nm), Cytophaga-Flavobacter-probe (488 nm), Calcofluor (380 nm)

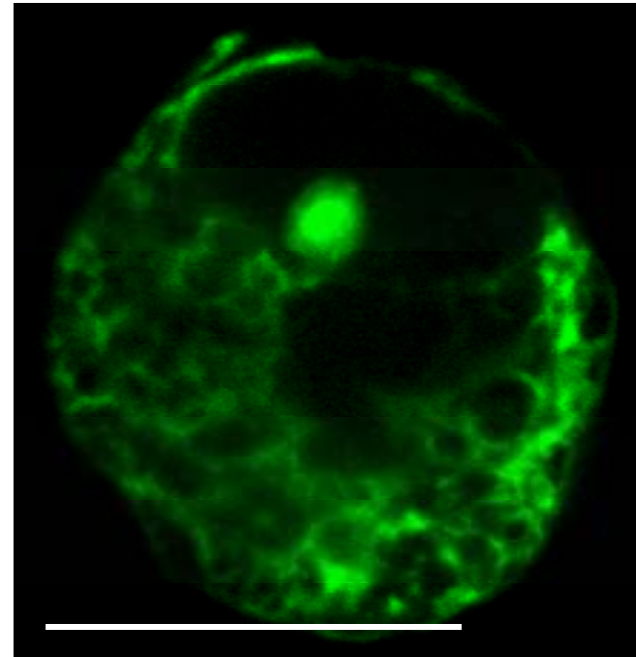
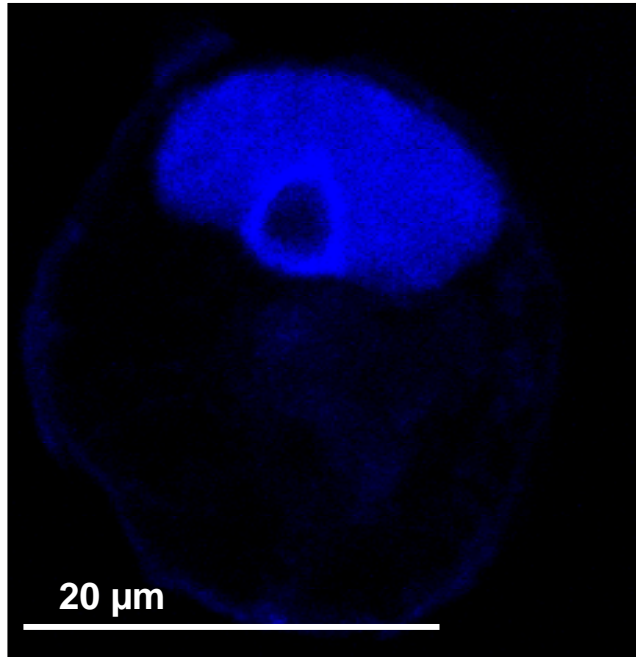


# Probe penetration in *Alexandrium fundyense*

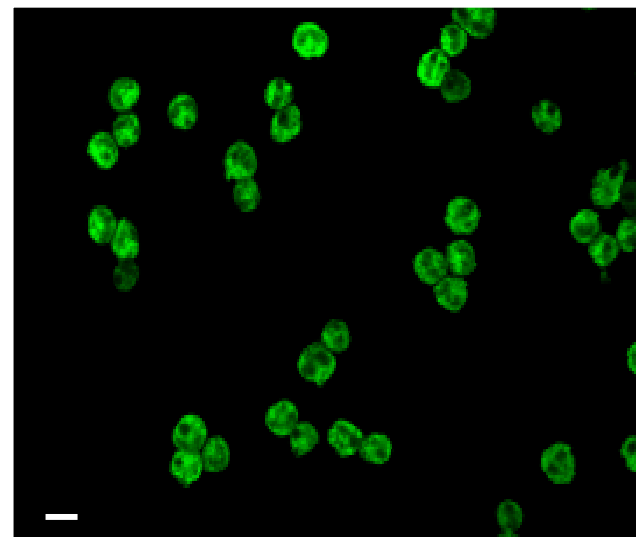
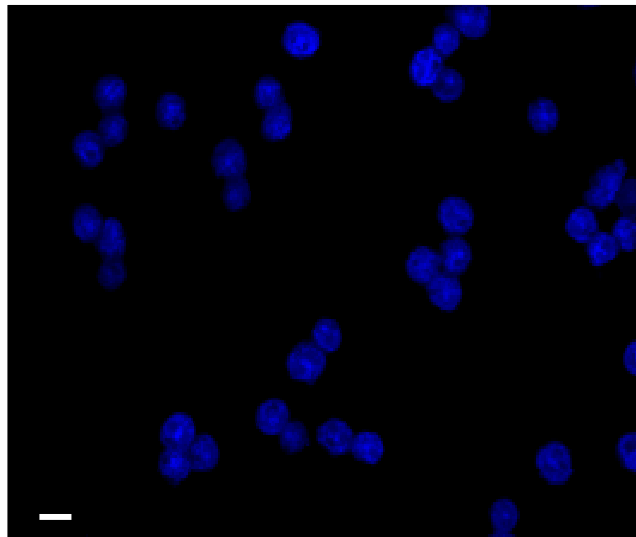
DAPI & calcofluor (380 nm)

Eukaryote-probes (488 nm)

Cyst stage



Vegetative stage

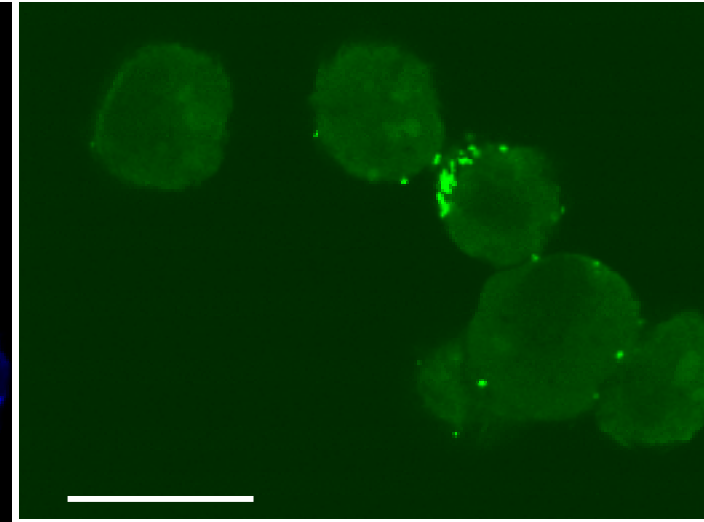
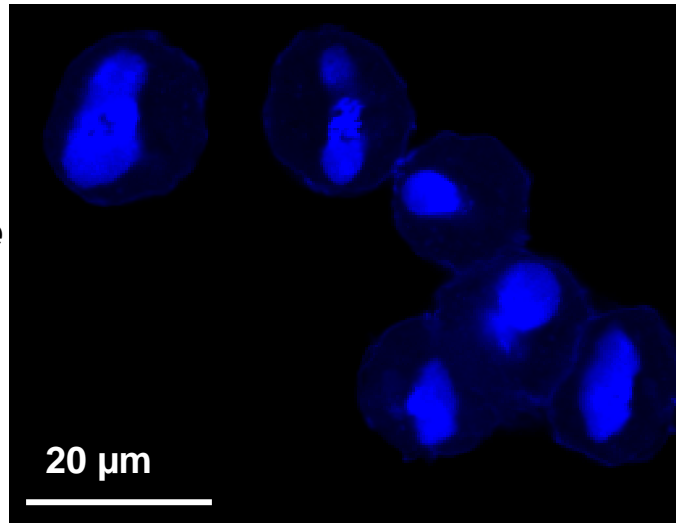


# Localization of intracellular bacteria

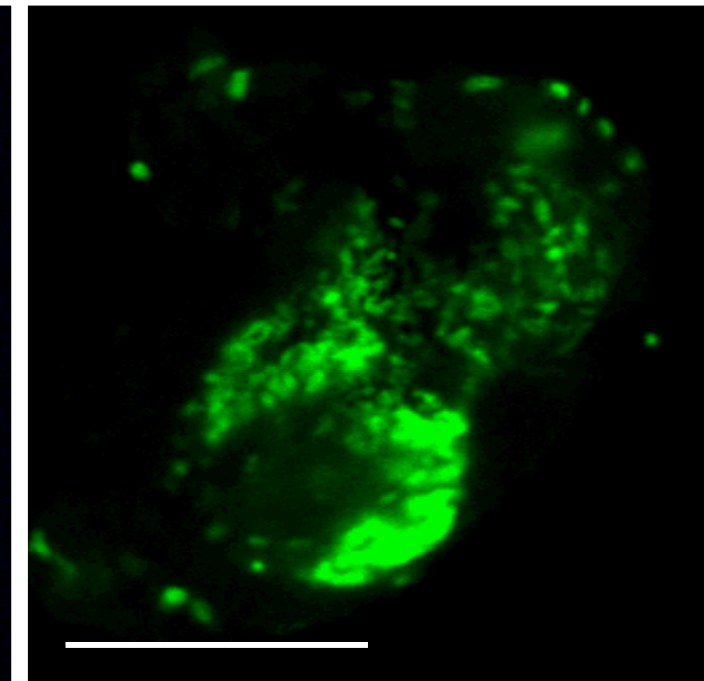
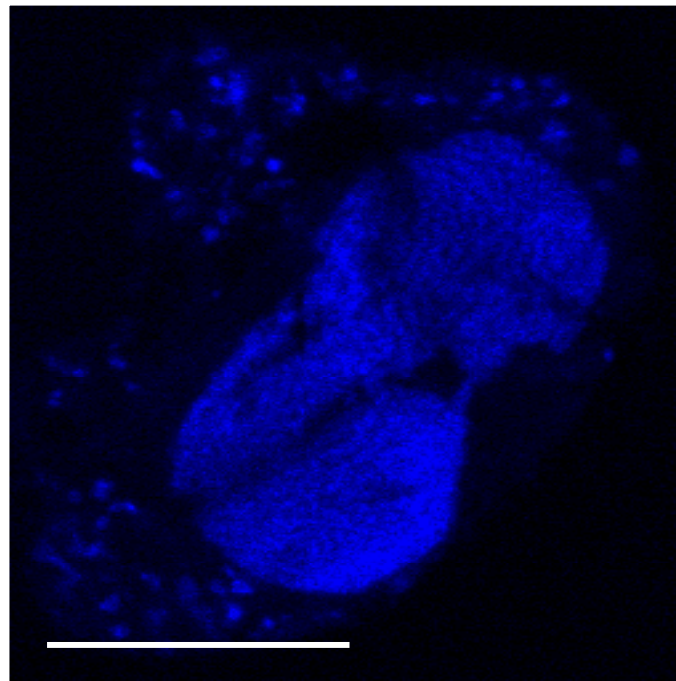
DAPI & calcofluor (380 nm)

Eubacteria-probes (488 nm)

*Alexandrium fundyense*

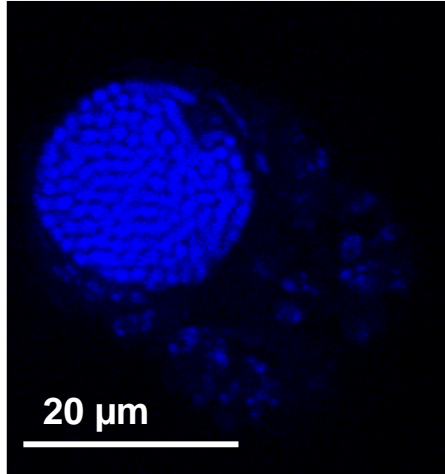


*Gyrodinium instriatum*

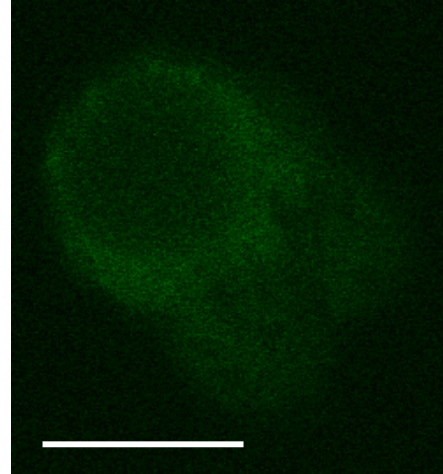


# Identification of intracellular bacteria in *G. instriatum*

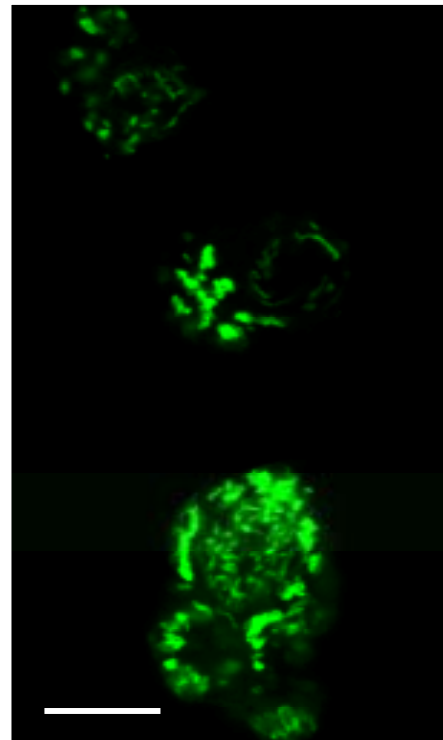
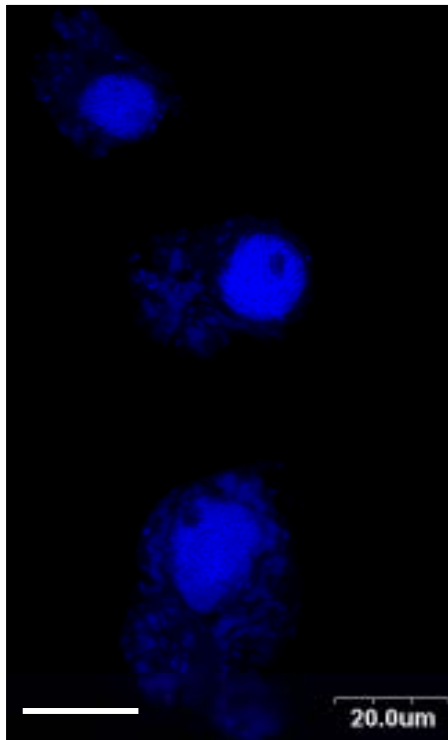
DAPI (380 nm)



probes (488 nm)



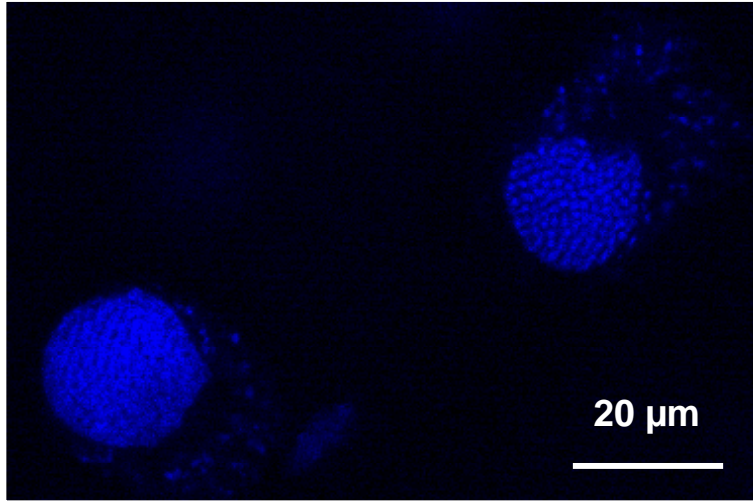
Alpha bacteria-probe



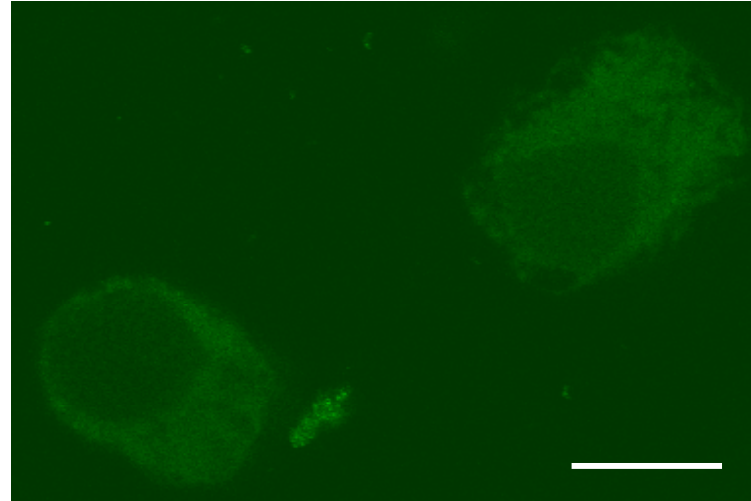
Beta bacteria-probe

# Identification of intracellular bacteria in *G. instriatum*

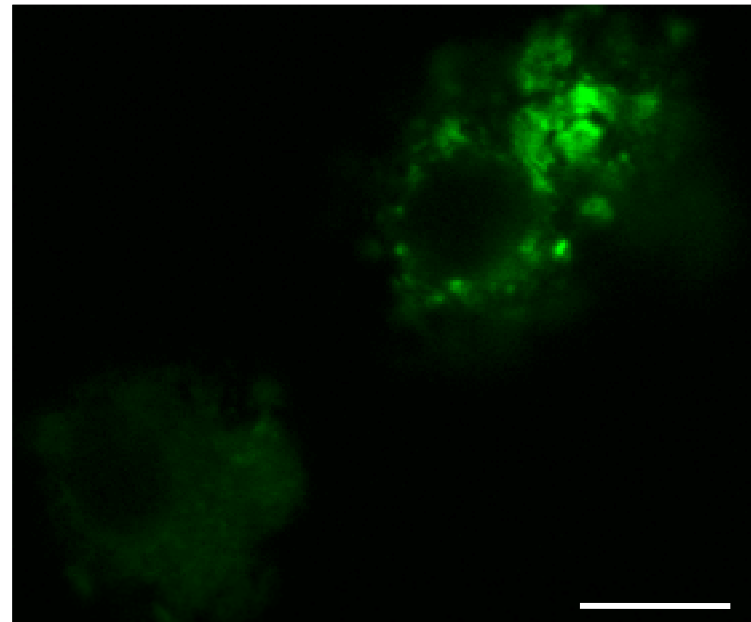
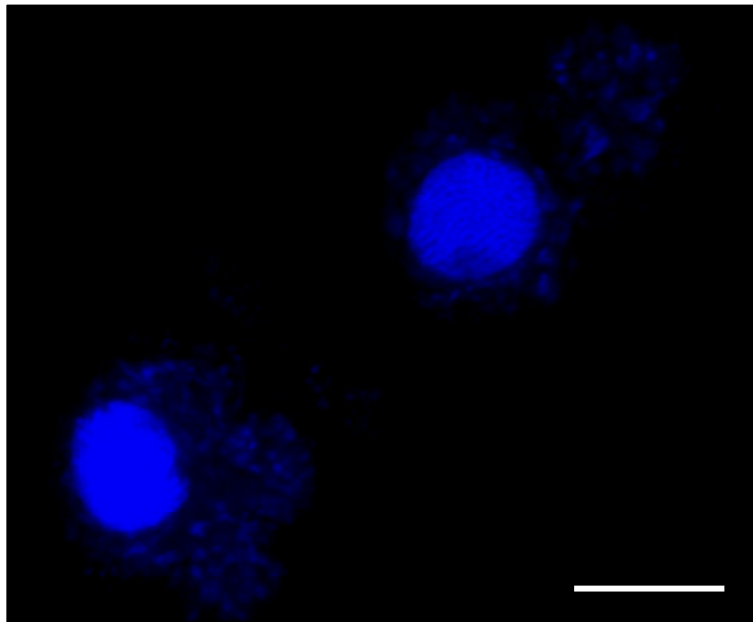
DAPI (380 nm)



probes (488 nm)



Gamma bacteria  
probe

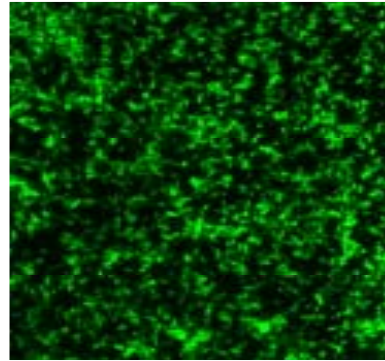
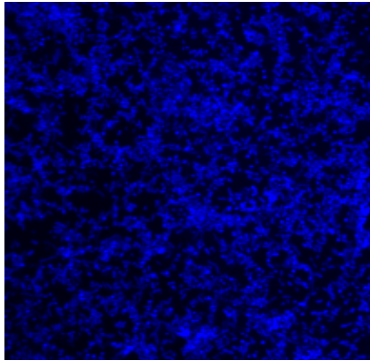


Cytophaga  
-Flavobacter  
bacteria probe

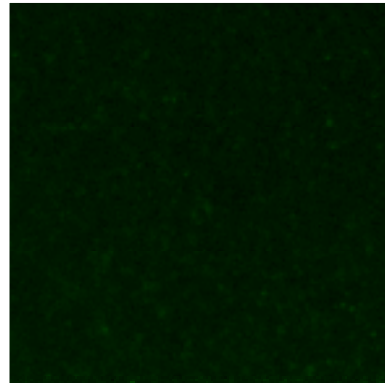
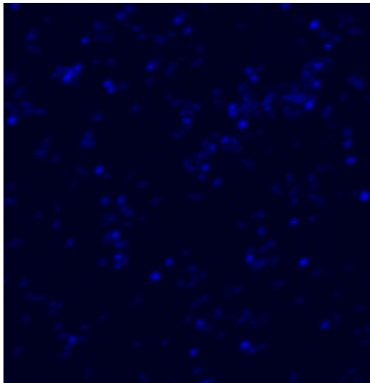
# Identification of free bacteria in *G. instriatum* culture

DAPI (380 nm)

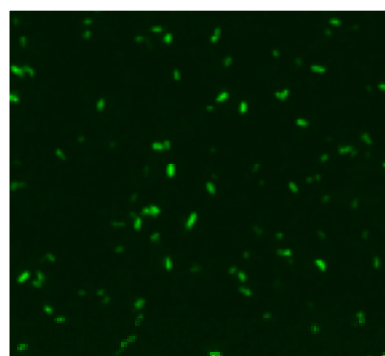
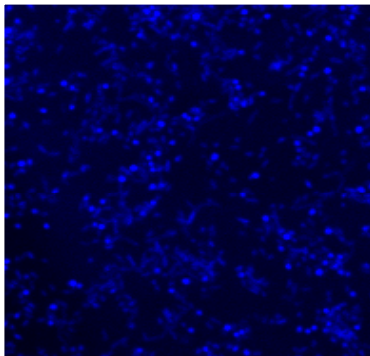
probes (488 nm)



Eubacteria-probe



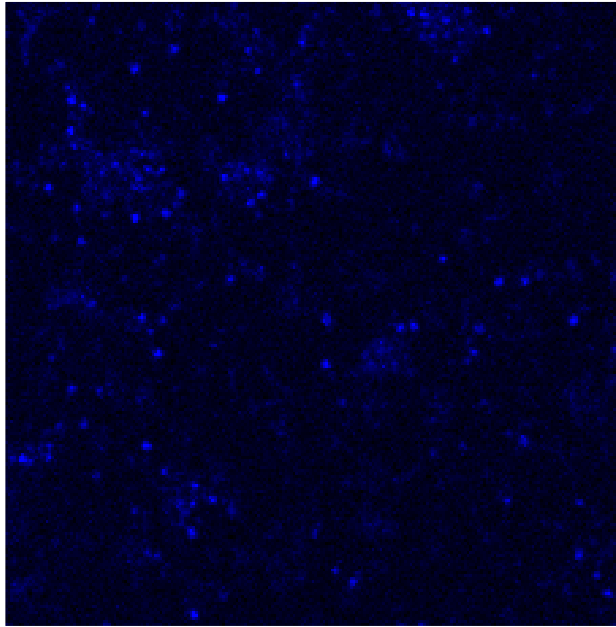
Alpha bacteria-probe



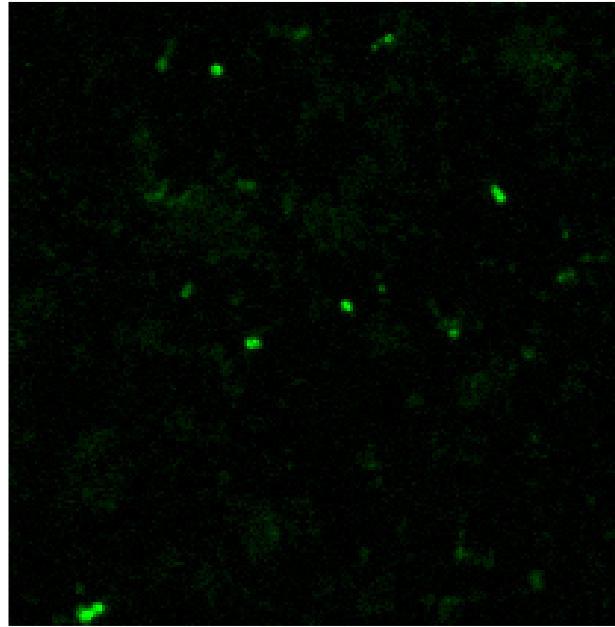
Beta bacteria-probe

# Identification of free bacteria in *G. instriatum* culture

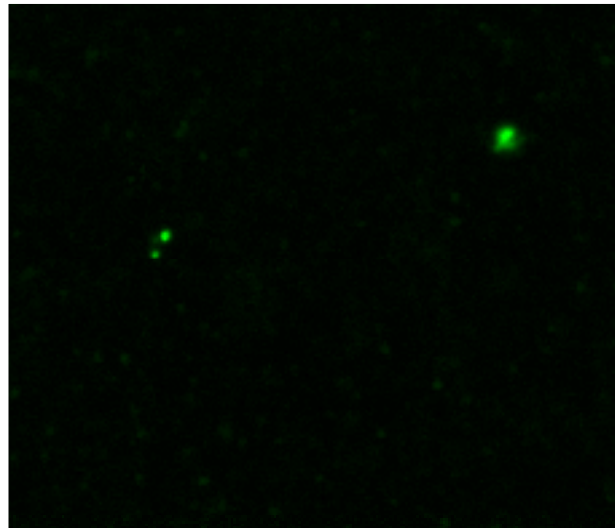
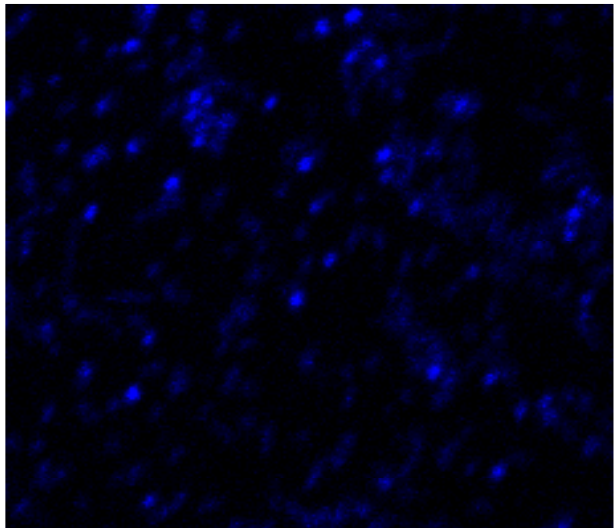
DAPI (380 nm)



probes (488 nm)



Gamma bacteria  
probe



Cytophaga-Flavobacter  
probe

# What are doing these bacteria inside the cytoplasm and the nucleoplasm of *Gyrodinium instriatum* ?

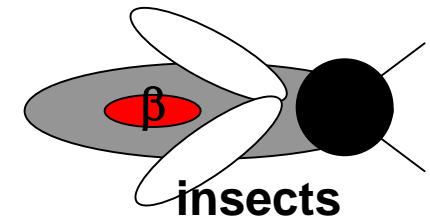
## 1- How common are beta-proteobacteria ?

### This study

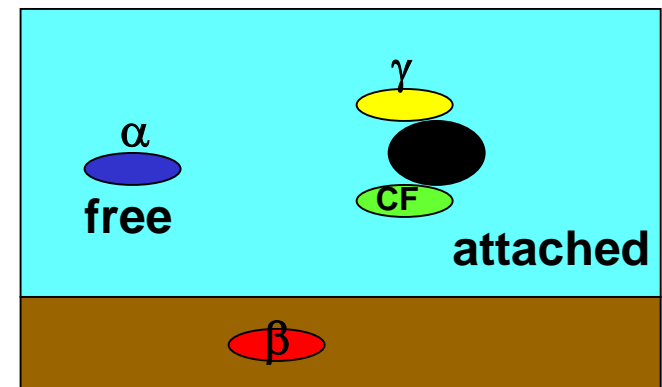


This result is surprising

### Literature information

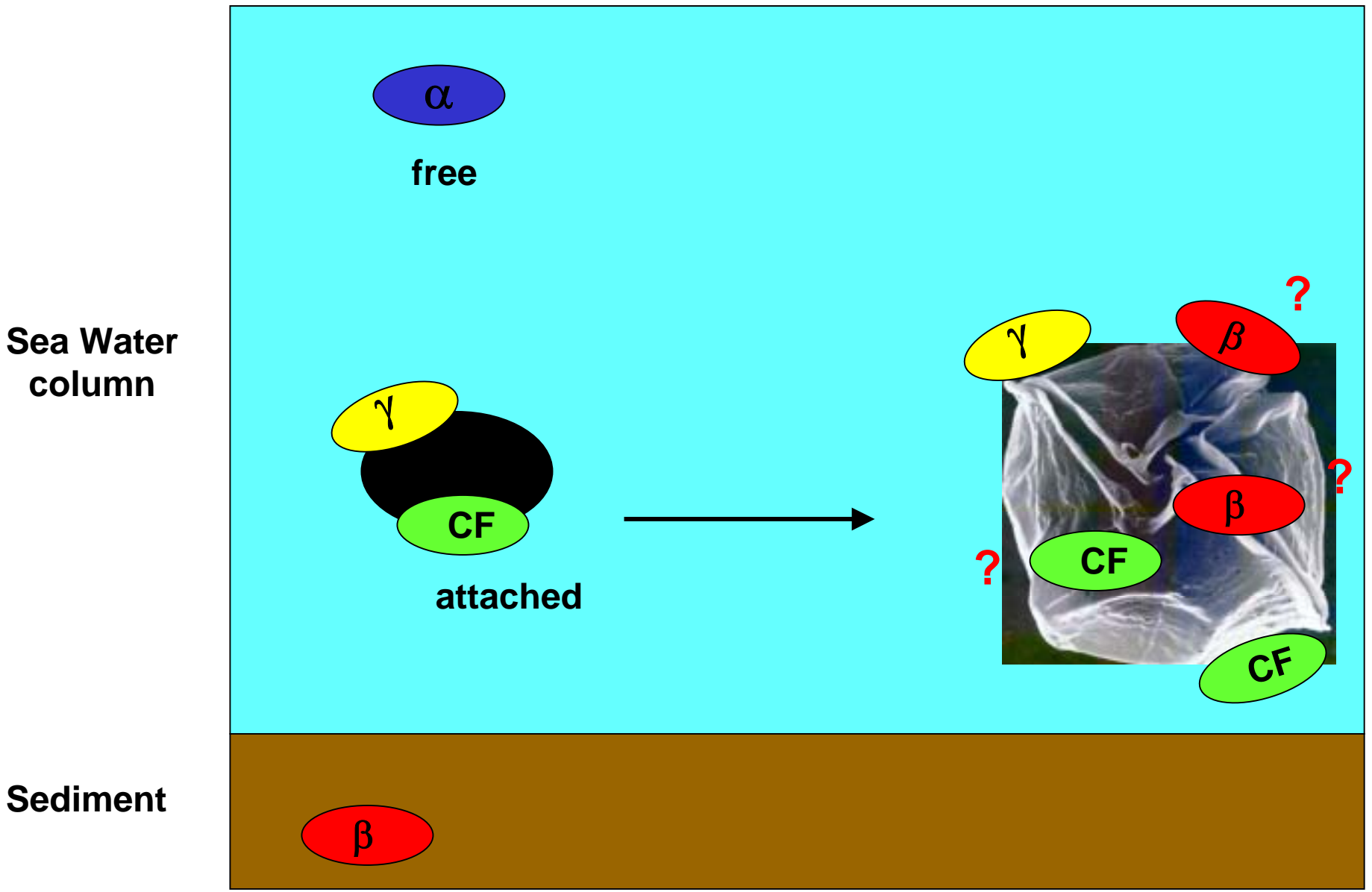


Sea Water  
column



Sediment

## 2- Why no alpha-proteobacteria were found in the culture ?



### 3- Why CF bacteria were found in the cytoplasm ?

#### This study



#### Literature information

CF

Develop on senescent phytoplankton

CF

Degrade complex macromolecules

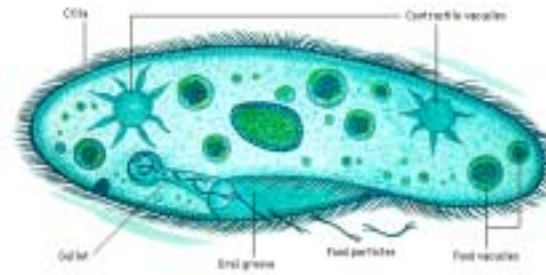


CF

Develop on few senescent dinoflagellates

## 4- What are doing beta-proteobacteria within the nucleus of the dinoflagellates ?

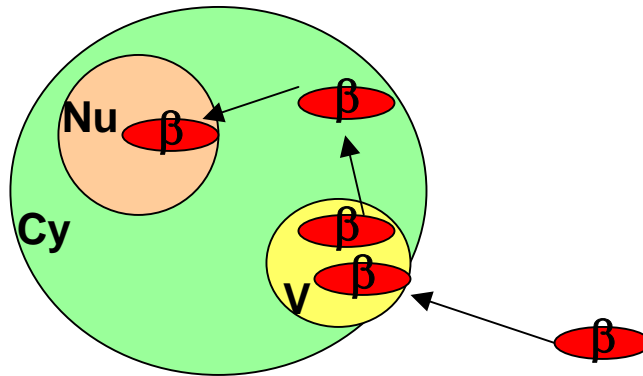
Literature (Görtz 1986)



(Ciliate, Alveolata)

*Paramecium sp.*

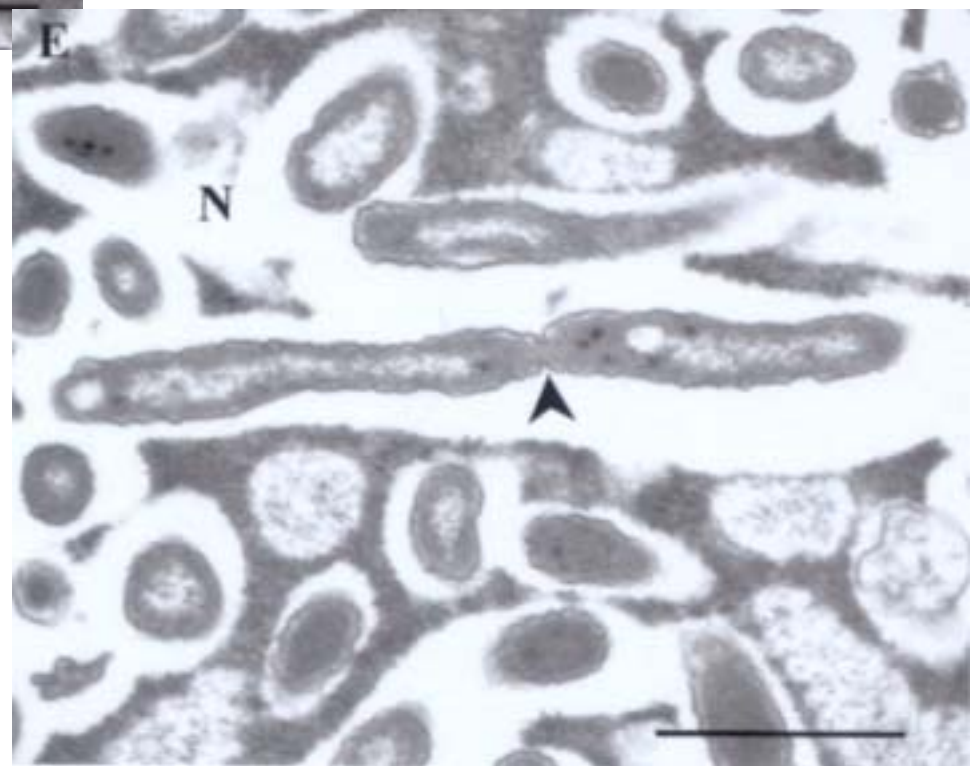
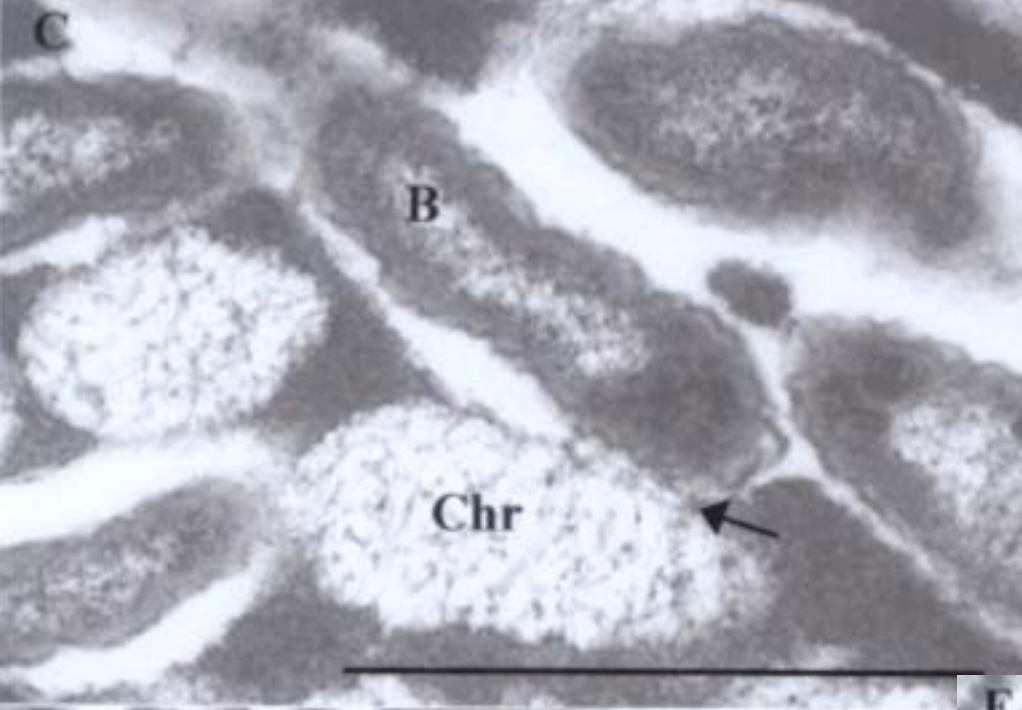
### Hypothesis



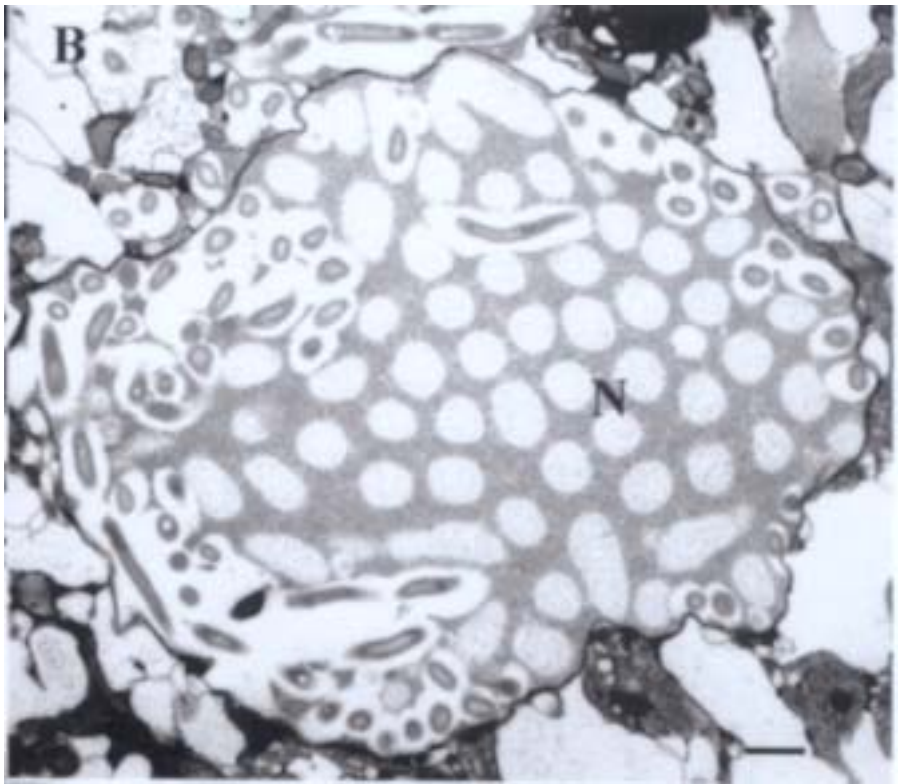
*G. instriatum* (dinoflagellate, Alveolata)

### Advantages to be in the nucleus :

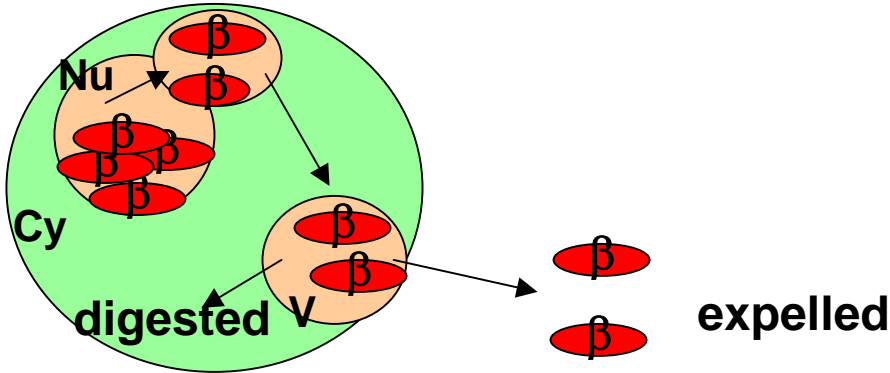
- The place is safe
- Food in nucleoplasm
- Spread between daughter cells



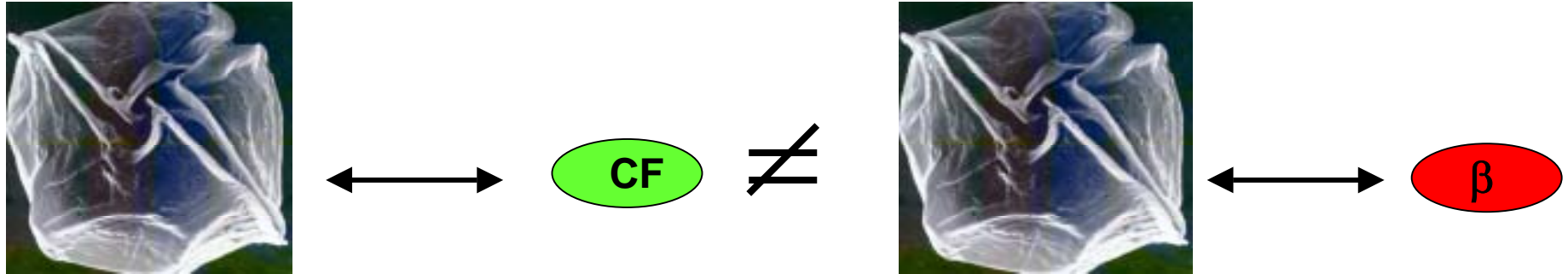
TEM observations on *G. instriatum*



Further hypothesis



## 5- Differences between the two kinds of relationships



- Few in dinoflagellates
- Endocyttoplasmic only
- Probably degrading bacteria

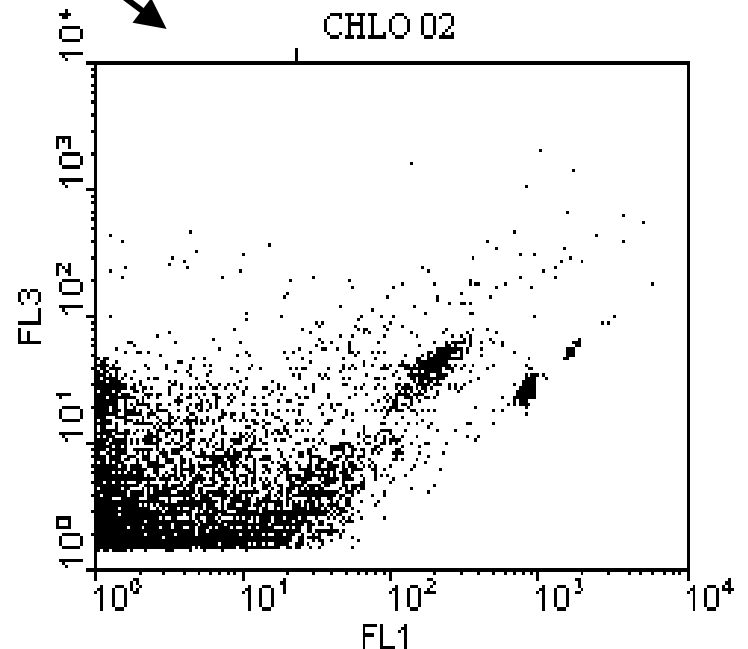
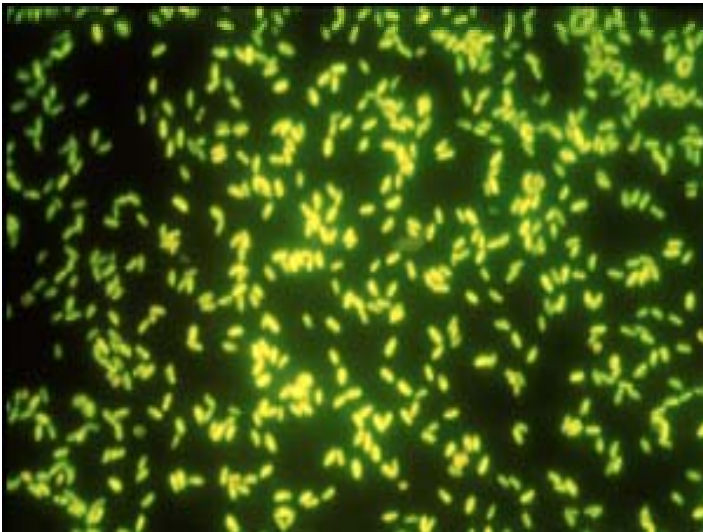
- All dinoflagellates
- Both endocyttoplasmic and endonuclear
- Observed since 18 yrs → « symbiotic » relationship

## Conclusion

**TSA-FISH and Confocal microscopy allow us to further characterise the relationship between the dinoflagellates or other phytoplankton cells with associated bacteria**

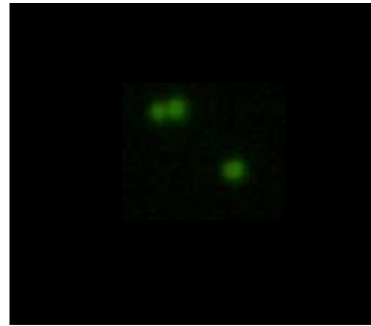
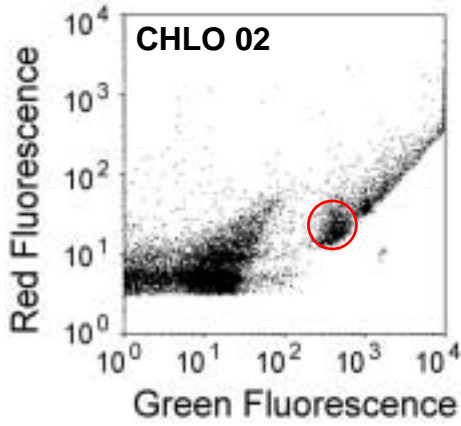
## Further applications

Identification and quantification of photosynthetic picoeucaryotes (< 3  $\mu\text{m}$ )

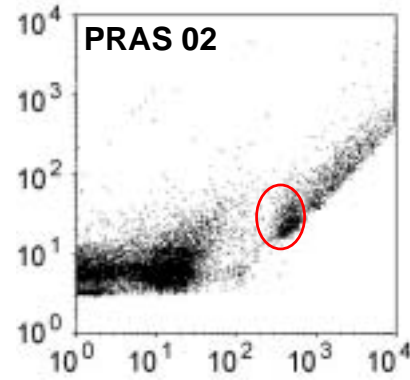




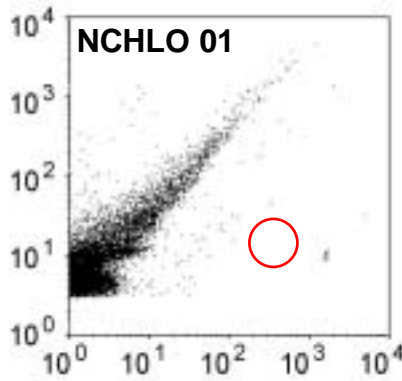
# Ostreococcus tauri SPECIFICITY



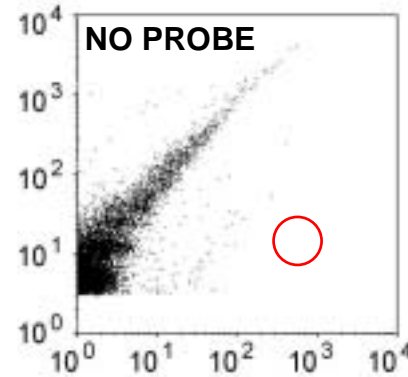
$$\bar{X} = 491$$



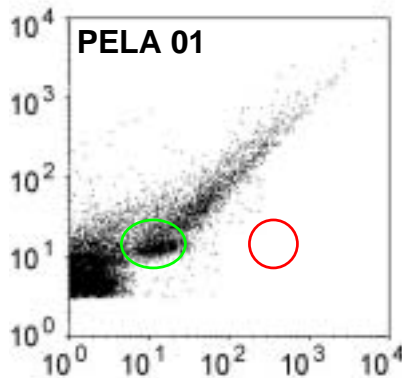
$$\bar{X} = 504$$



$$\bar{X} =$$



$$\bar{X} =$$

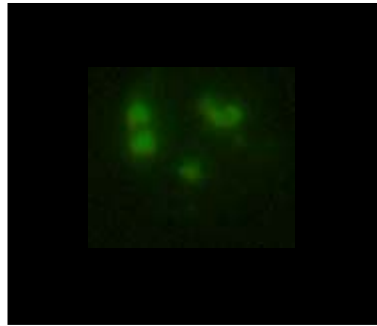
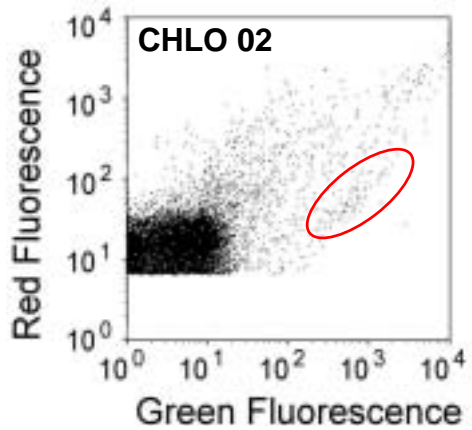


$$\bar{X} = 16$$

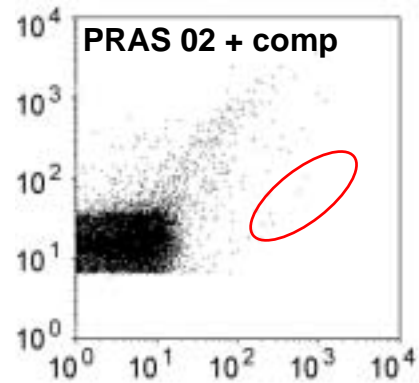
Range of fluorescence ratio:

31 - 32

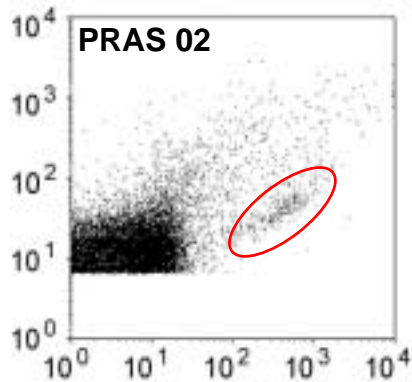
# ASTAN 12/04/2001 SPECIFICITY



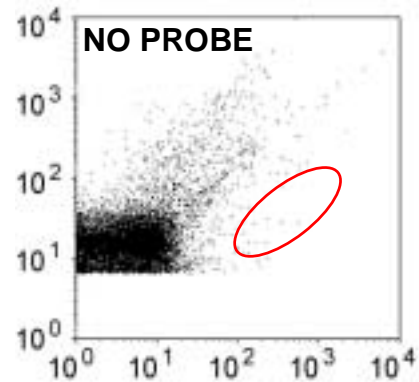
**N = 254**  
 **$\bar{X}$  = 664**



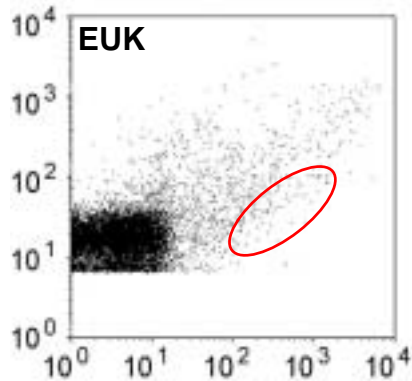
**N = 10**  
 **$\bar{X}$  = 393**



**N = 680**  
 **$\bar{X}$  = 591**



**N = 4**  
 **$\bar{X}$  = 262**



**N = 187**  
 **$\bar{X}$  = 667**

# Identification of attached bacteria in mixed phytoplankton

Eubacteria-probe (568 nm), Cytophaga-Flavobacter-probe (488 nm)

