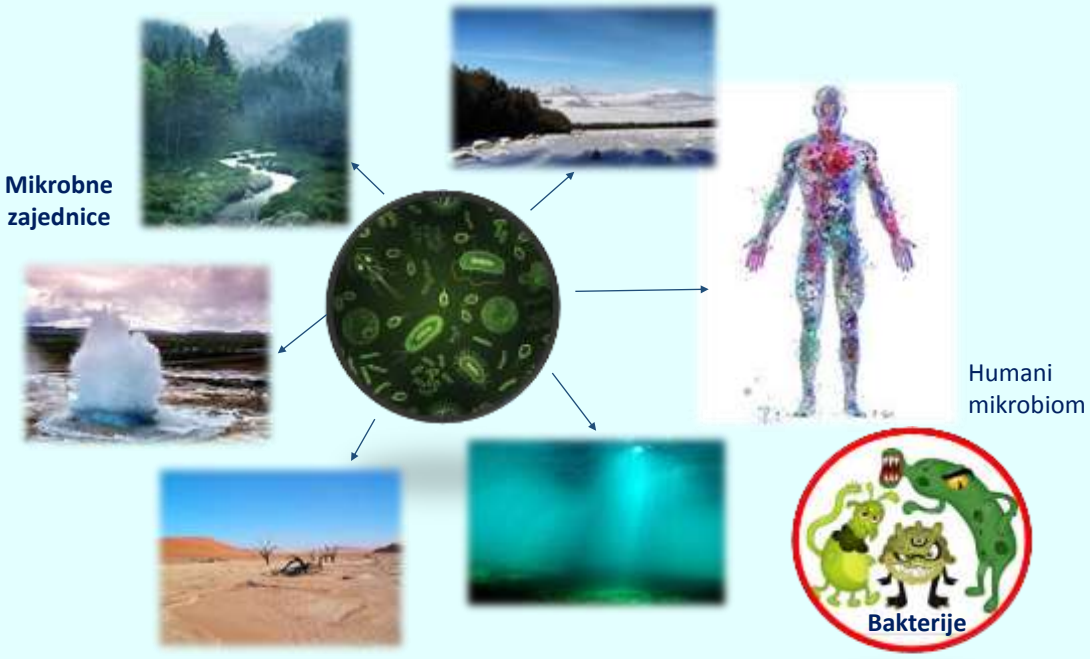
# I SITNO JE BITNO: *Prochlorococcus marinus* Najsitniji i najabundantniji fotosintetski organizam na Zemlji

Ivana Bošnjak

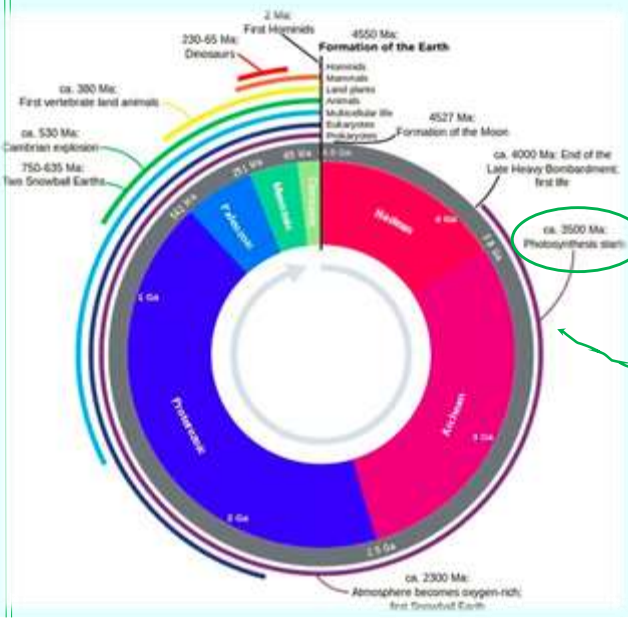
HBoD, 29. siječnja 2015.

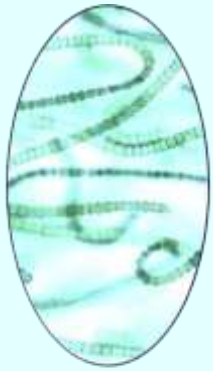
**Svugdje prisutni mikroorganizmi….**



## Koljeno: Cijanobakterije (lat. *Cyanobacteria*)

[grčki *kyanós* = plavi]



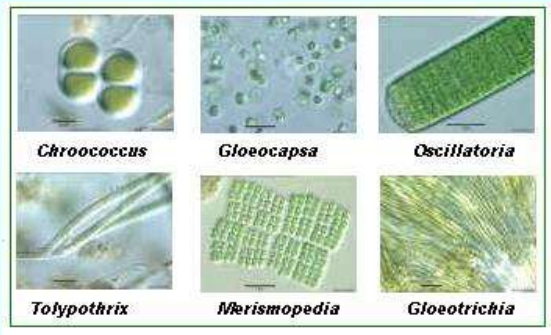



**Modrozelene alge**

Fotosinteza!

**Pretkambrij (proteozoik) = 3.5 miljardi godina**

## Cijanobakterije (lat. *Cyanobacteria*)




2000 vrsta svrstanih u 150 rodova

- ✓ Jednostanični, kolonijalni ili višestanični oblici
- ✓ Sluzavi želatinozni omotač na površini → štiti ih od isušivanja
- ✓ Vlažna staništa:
  - Na i u tlu, površina stijena
  - **Vodene: slatkovodne i morske**

*Prochlorococcus marinus*

20% kisika u Zemljinoj atmosferi

Cvijetanje cijanobakterija blizu Fi
(južni Tihi ocean)

## Plankton

**1970-tih...**

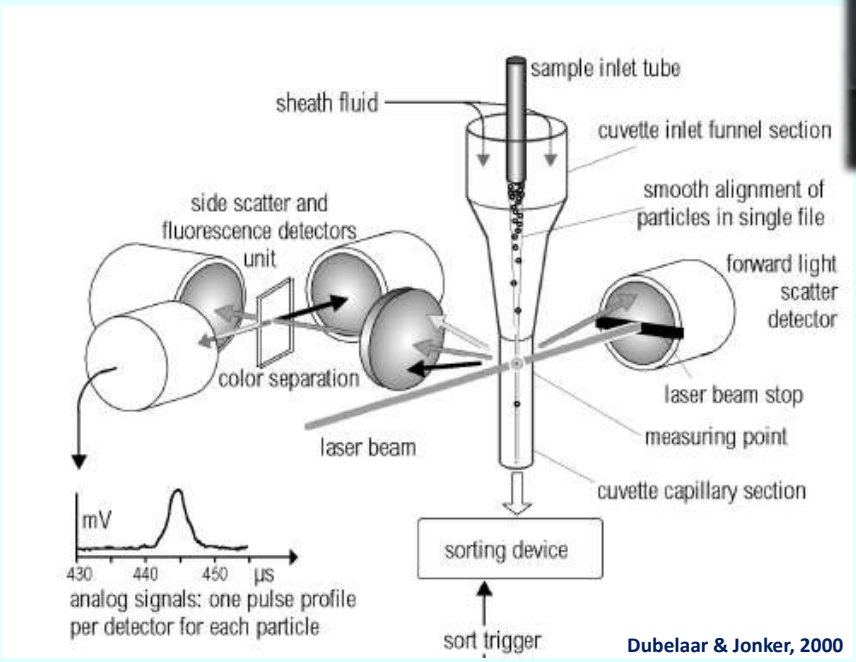

**Elektronski mikroskop**

## Istraživanja ocenaskog pikoplanktona

**70-tih 80-tih godina 20. stoljeća**
**Sjeverni Atlantik**

**uzorci → obrađuju se u laboratoriju!**

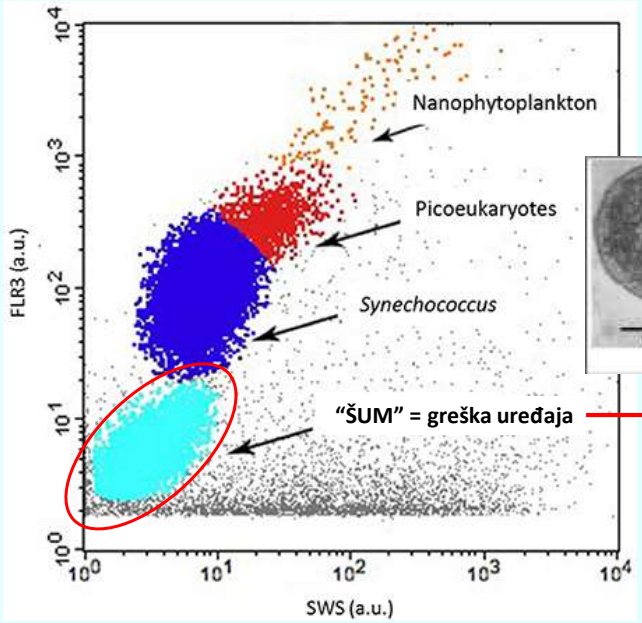
## Krajem 1970-tih → **Protočni citometar**

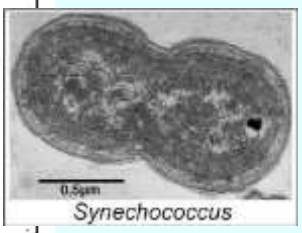



Dubelaar & Jonker, 2000

**1 – 5 min po uzorku**
**1000 stanica / sekundi**

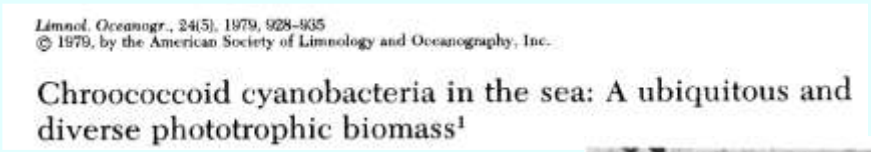
## Protočni citometar → **Analiza pikoplanktona**




- ✓ < 0.8 μm
- ✓ i u uzorcima uzetim sa dna epipelagijala (400 m)

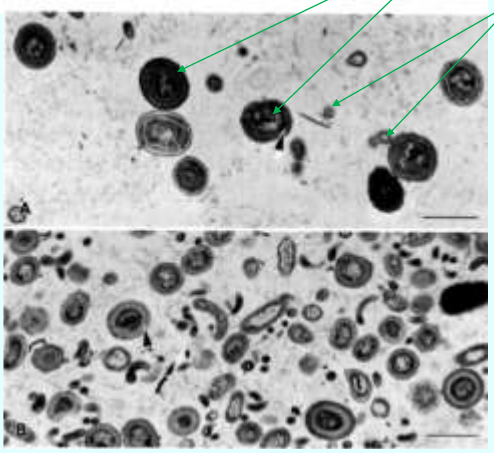
## Johnson, P. W. & Sieburth, J. M. (1979.)



*Limnol. Oceanogr.*, 24(5), 1979, 928–935
© 1979, by the American Society of Limnology and Oceanography, Inc.

Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass[1]

- ✓ **„Tip II" stanice →** slične cijanobakteriji *Synechococcus*
- ✓ Stanice nemaju pigment fikoeritrin
- ✓ Nije ih moguće uzgojiti u kulturi




**Elektronski mikrograf**

**Sallie W. Chisholm** (Massachusetts Institute of Technology)
**Robert J. Olson** (Woods Hole Oceanographic Institution)

Deep Sea Research Part A. Oceanographic Research Papers
Volume 32, Issue 10, October 1985, Pages 1273–1280

**Marine phytoplankton distributions measured using shipboard flow cytometry**

R.J. Olson*, †, D. Vaulot*, ‡, S.W. Chisholm*


letters to nature

**A novel free-living prochlorophyte abundant in the oceanic euphotic zone**

SALLIE W. CHISHOLM, ROBERT J. OLSON, ERIK R. ZETTLER, RALF GOERICKE, JOHN B. WATERBURY & NICHOLAS A. WELSCHMEYER

**1988.**

Arch Microbiol (1992) 157: 297–300
Archives of Microbiology
© Springer-Verlag 1992

*Short communications*

***Prochlorococcus marinus*** **nov. gen. nov. sp.: an oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b***

Sallie W. Chisholm[1], Sheila L. Frankel[1], Ralf Goericke[2], Robert J. Olson[2], Brian Palenik[1,2,3], John B. Waterbury[2], Lisa West-Johnsrud[1], and Erik R. Zettler[2]

**1992.**

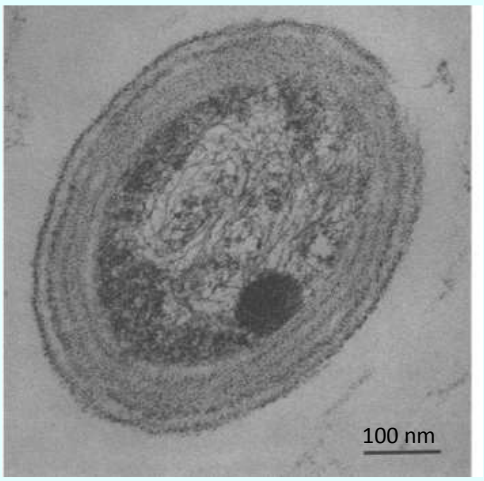
## **Sallie W. Chisholm** (Massachusetts Institute of Technology, USA)

*"It is difficult to describe the thrill of studying Prochlorococcus. The name alone is enough to stop a conversation. Far from being tedious, studying this extraordinary little cell is like opening a present every day. It is a gift, and a responsibility. "*

Unveiling *Prochlorococcus*


National Medal of Science
Alexander Agassiz Medal (2010)

## Klasifikacija vrste




**Elektronski mikrograf**

| | |
|---|---|
| **Carstvo:** | Bacteria |
| **Koljeno:** | Cyanobacteria |
| **Red:** | Synechococcales |
| **Porodica:** | Synechococcaceae |
| **Rod:** | *Prochlorococcus* |
| **Vrsta:** | ***Prochlorococcus marinus*** Chilsom et al., 1992 |

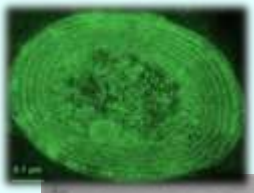
grčki ***Pro*** = ishodišni, prvi

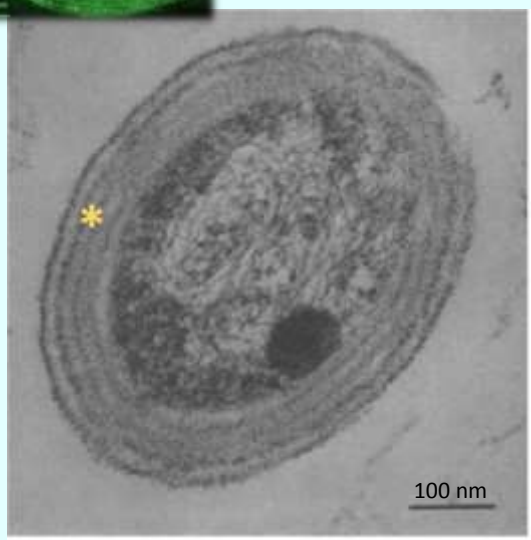
grčki ***chloros*** = zelen

višejezično značenje ***coccus*** = bobica

**"Little greens"**
**Chilsom et al., 1992**

## Opis vrste *Prochlorococcus marinus*






- ✓ Veličina stanice: **0,5 do 0,7 µm**
  - ➢ ovisi o uvjetima u okolišu (npr. sumrak ili izlazak sunca na ekvatoru, dubina, itd.)
- ✓ Oblik stanice: **izdužen**
- ✓ Volumen stanice: **0.1 µm³**
- ✓ Citoplazma stanice: 1 kružna DNA (**nukleoid**) i **karboksisomi**
- ✓ **Tilakoidni sustav:** 2 ili 4 tilakoide, prilježu uz staničnu membranu (*), paralelno smještene

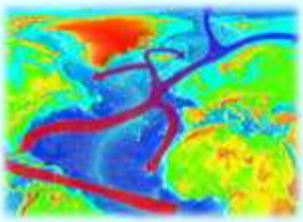
**Chilsom et al., 1992**

## Kultivirani sojevi

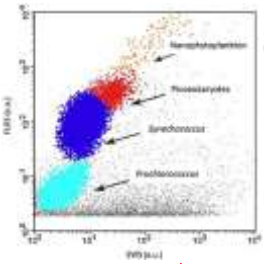
*Prochlorococcus sp*. SS120
120 m

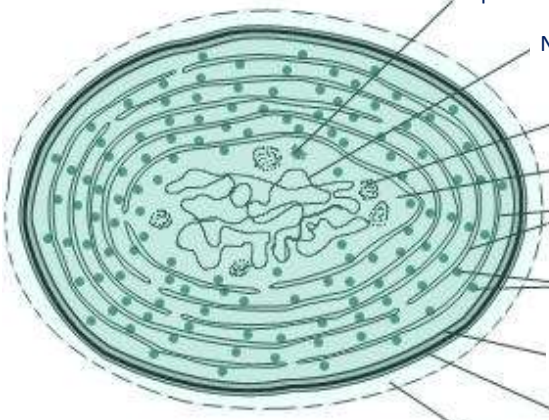
*Prochlorococcus sp*. MED
površinski sloj


*Prochlorococcus sp*. MIT 9313
Golfska struja, dubina 135 m



# Pigmenti





Lipidne kapljice

Nukleoid

Karboksisomi

Ribosomi

Tilakoidni sustav

**Klorofil-protein kompleks = Pcbs**

Pigmenti: **divinil klorofil a (Chl a2) i**

**divinil klorofil b (Chl b2)**

Stanična membrana

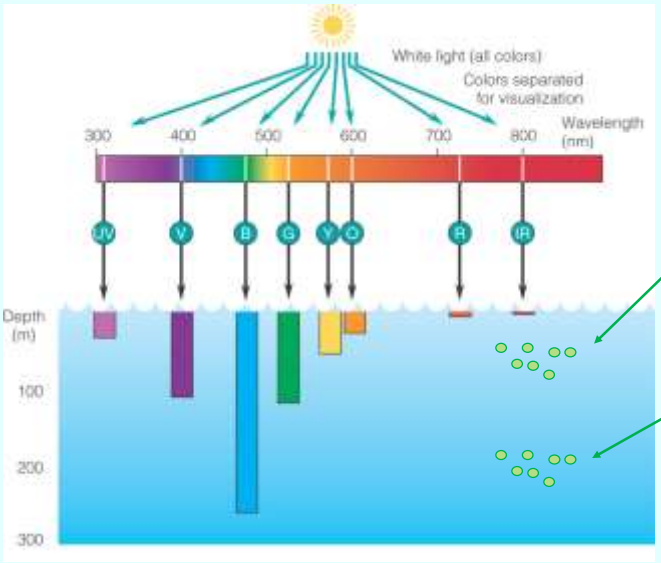
Stanična stijenka

Želatinozni omotač

~~Nema fikobilisome!~~

Ostali pigemnti:
Zeaksantin
α-karoteni
Klorofilu c-sličan pigment

**divinil klorofil b (Chl b2)** → omjer Chl b2/a2




## Ekotipovi:

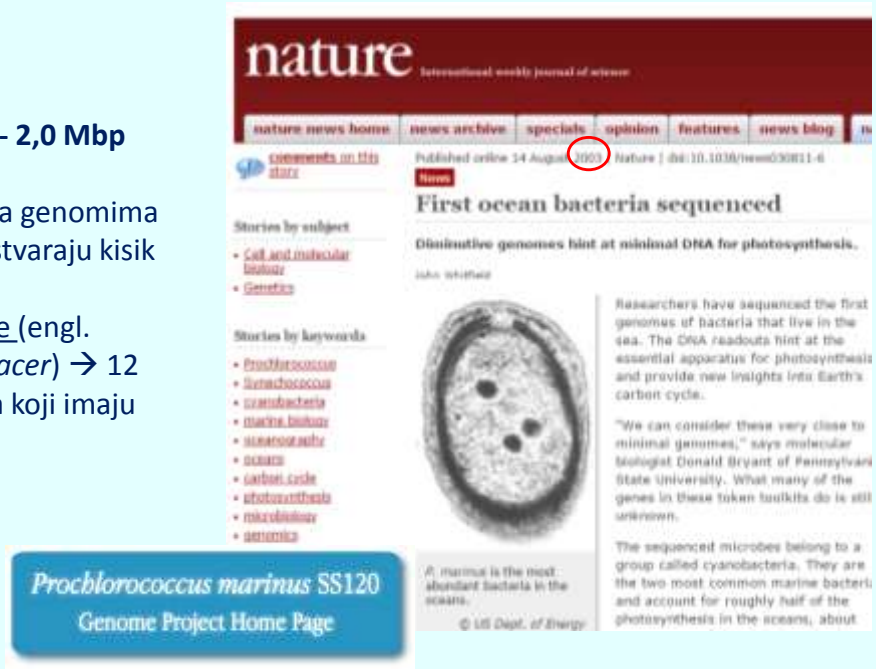
**HL** (od engl. *high light*) =
"jakog svjetla"
→ niski Chl b2/a2

**LL** (od engl. *low light*) =
"slabog svjetla"
→ visoki Chl b2/a2

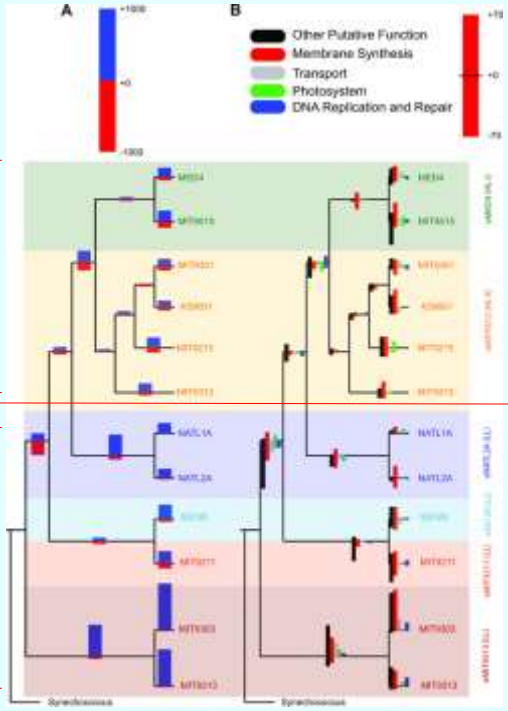
Iskorištenje ugljika, dušika, fosfora i željeza iz okoliša

## Genom

- ✓ Prosječna veličina: **1,9 - 2,0 Mbp**
- ✓ najmanji u usporedbi sa genomima drugih prokariota koji stvaraju kisik
- ✓ 16S-23S rDNA ITS regije (engl. *internal transcribed spacer*) → 12 *Prochlorococcus* sojeva koji imaju različite genotipove




## 12 genotipova:




**6 HL ekotip**
(niski omjer Chl b2/a2)

**6 LL ekotip**
(visoki omjer Chl b2/a2)

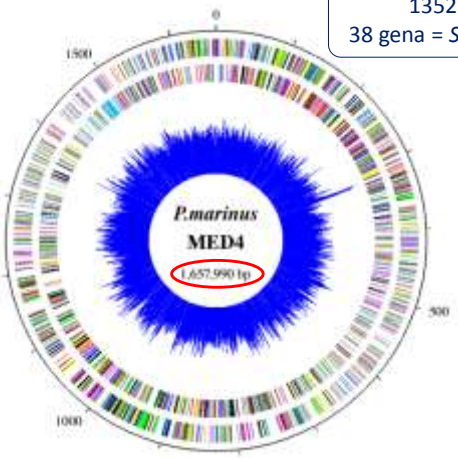
**Svi imaju 1273 identičnih gena → "*the core*"**

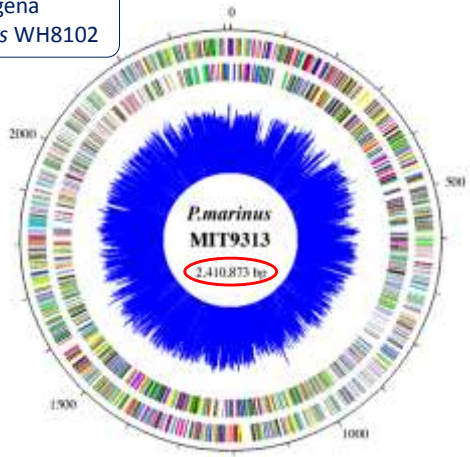
Ketler et al., ***PLoS genetics*, 2007.**

**HL predstavnik:**

1352 zajedničkih gena
38 gena = *Synechococcus* WH8102

**LL predstavnik:**



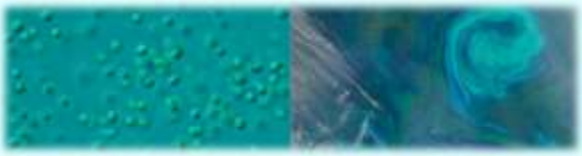




- kodira za 1716 proteina
- G + C sastav = 30,8%

- kodira za 2275 proteina
- G + C sastav = 50,74%

gubitka određenih gena uključenih u put popravka DNA molekule

## Ekologija



✓ Eufotična i difotična zona: **200 – 400 m**

✓ Koloniziraju ekstremno **oligotrofna područja → velika površina *VS*. mali volume stanice (V = 0.1 µm$^3$)**

✓ Stanična dioba: **1x dnevno →** najbrojniji za vrijeme ljeta i jeseni

✓ Temperaturni raspon: **10 – 33°C**

✓ **LL ekotipovi** = dublji slojevi

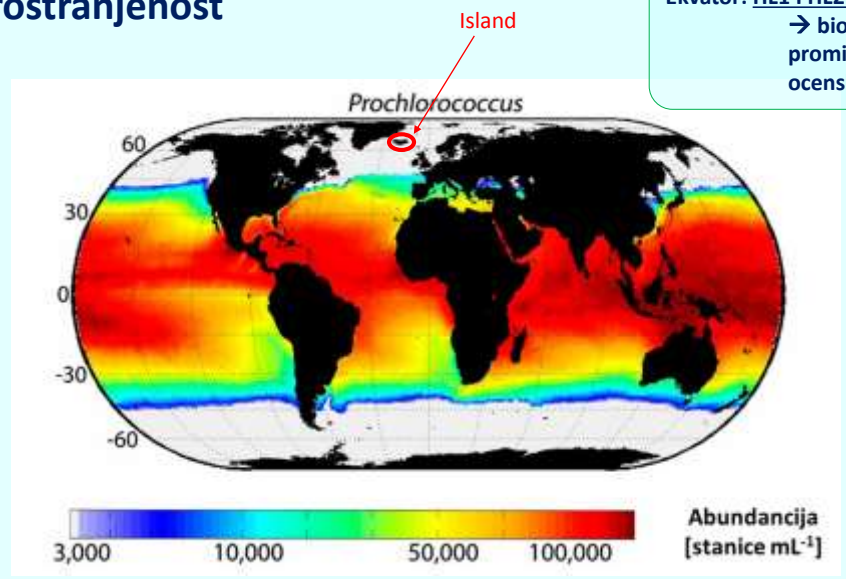
✓ **HL1** i **LL1** = mogu preživjeti u miješanim, hladnijim vodenim masama

**1 mL morske vode → više od 100000 stanica**

**prosječna godišnja brojnost = 2,8 - 3 oktiljuna (~10$^{27}$)**

## Rasprostranjenost

Ekvator: HL1 I HL2 ekotipovi
→ bioindikatori praćenja promijene temperature ocenske morske vode



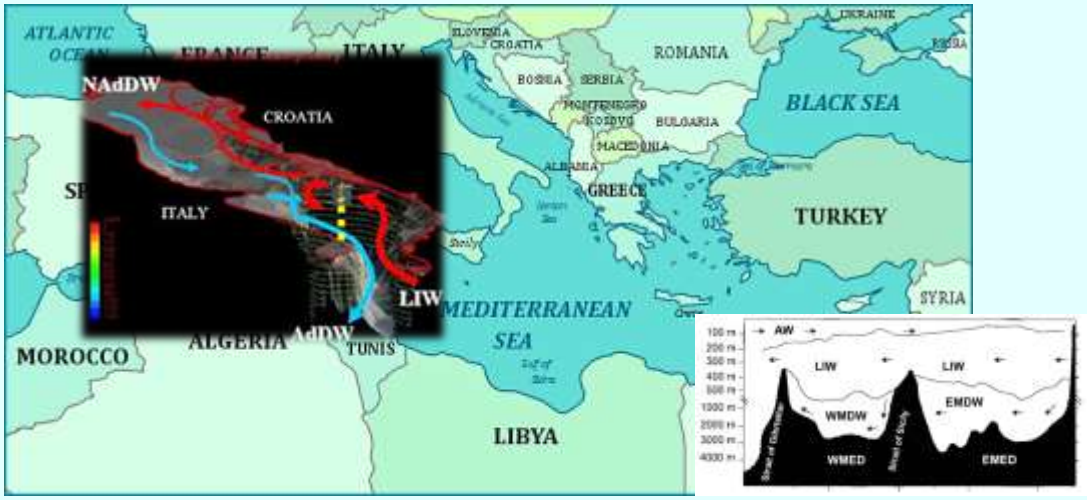

Flombaum et al., 2013.

## *Prochloroccocus* u Jadranskom moru

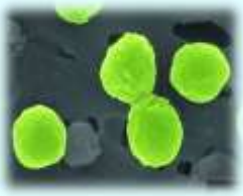
- ✓ 2009. godine = detektiran metodom protočne citometrije (Radić et al., 2009)
- ✓ 2011. godine = HPLC metodom detektiran pigment divinil klorofil a (Chl a) (Šilović et al., 2011)
- ✓ 0 do $10^4$ stanica po mL morske vode uz obalu
- ✓ $10^3$ do $10^4$ stanica po mL morske vode u dubljim dijelovima

***Prochlorococcus* → detekcija ulaska LIW i njeno stujanje u Jadranskom moru**

**Levantinska intermedijarna struja** (**LIW**, engl. *Levanitine Intermediate Water*)




topla (>14°C) i slana (>38.75 ‰) morska masa na dubini od 40 do 600 metara



## *Prochlorococcus marinus*

- ✓ Mikrobni pikoplanktonski organizam otkriven tek prije 25 godina
- ✓ U oceanima u triljunskim količinama → "***Prochlorococcus* federation**"
- ✓ Zaslužan za produkciju 20 % kisika na Zemlji i → "pluća" oceana
- ✓ Zaslužan za 50 % primarne produkcije u biosferi (fiksacija $CO_2$)
- ✓ DOMINANTAN i NAJSITNIJI fotosintetski organizmi na Zemlji

**Cijanobakterija – superheroj!**

**Zahvale:**

Financiranje projekta:


**Zrinka Ljubešić**

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**Laboratorij za alge**

**BIOTA tim**