Ecological advantages from light adaptation and heterotrophic-like behavior in *Synechococcus* harvested from the Gulf of Trieste (Northern Adriatic Sea)

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Abstract

A preliminary study was carried out on a picocyanobacterial mixed culture harvested from the Gulf of Trieste (Northern Adriatic) and identified as *Synechococcus* spp. both by transmission electron microscopy observations, biliprotein composition and molecular analyses. Absorption and fluorescence spectra revealed phycourobilin and phycoerythrobilin chromophores, suggesting the presence of both CU- and C-phycoerythrin, besides phycocyanobilin chromophores typical for phycocyanins and allophycocyanins. Both biliprotein analyses and molecular identification indicated the presence of at least two *Synechococcus* subgroups presumably differing either in phycoerythrin type or in physiological traits. Among the exoenzymatic activities acting on different substrates, only aminopeptidase showed high hydrolysis rates and the uptake of organic molecules was positive for leucine but not for thymidine. The protein carbon mobilized was high compared with the leucine incorporation rates, resulting in low percentages of newly mobilized carbon utilized by cultures. The organic carbon incorporated as leucine was compared with the photosynthetically produced one, and the balance between the phototrophic- and heterotrophic-like processes was c. 3:1. Our findings suggest that the *Synechococcus* heterotrophy plays an important role in cell’s metabolism, and that the photoheterotrophic behavior, together with their chromatic adaptation capability, might represent the key for the absolute dominance of this genus in the Adriatic Sea.

Introduction

The phycoerythrin-rich cyanobacteria of the *Synechococcus* type (Olson *et al*., 1990; Sidler, 1994; Ting *et al*., 2002; Six *et al*., 2005), which inhabit the world’s coastal and oligotrophic oceans at high concentrations (generally $10^6–10^7$ cells L$^{-1}$), are also largely dominant within the autotrophic picoplankton (APP) community of the Northern Adriatic transitional, coastal and offshore waters (Bernardi Aubry *et al*., 2006; Paoli & Del Negro, 2006; Paoli *et al*., 2007). In this semi-enclosed basin, the APP, ranging between $10^5$ and $10^7$ cells L$^{-1}$ depending mostly on seasons (see Paoli *et al*., 2007 and references therein), contributes a percentage of 30–31% (with peaks up to 70%) to total phytoplankton carbon biomass (Bernardi Aubry *et al*., 2006), and is responsible for a substantial, sometimes dominant, fraction of the primary production (see Magazzù & Decembrini, 1995 for a review). The now well-recognized importance of these organisms in marine carbon cycling has led to greatly increased interest in the physiology, ecology and molecular biology (Scanlan & West, 2002) of the genus. At least some *Synechococcus* strains, similar to several cyanobacterial genera, are known to acclimate to light quality through a process traditionally called complementary chromatic adaptation (Tandeau de Marsac, 1977; Talarico, 1996; Talarico & Maranzana, 2000; Grossman, 2003). These ‘chromatically adapters’ synthesize maximally phycocyanin in red light and phycoerythrin with high or low phycourobilin/phycoerythrobilin chromophore ratios in blue and green light respectively. According to Palenik (2001), the ability to adapt chromatically may help explain the global distribution of *Synechococcus*. Research has so far focused on nutrient...
uptake, photosynthesis, motility and cell cycle behavior, but the *Synechococcus* photoheterotrophy has generally been considered to be of scarce ecological importance (see Zubkov et al., 2003 and references therein). We believe that this physiological feature should not be underestimated when modeling marine ecosystems as this might lead to significant differences in balancing the carbon cycle, particularly in nearly oligotrophic areas like the Gulf of Trieste, where community respiration may exceed the generally low primary productivity (Fonda Umani et al., 2007). Recently, Zubkov et al. (2003) stated that in oceanic oligotrophic waters the dominance of the genus *Prochlorococcus*, which consumes organic as well as inorganic nitrogen pools, may result in an overestimation of about 30% of bacterial secondary production and hence of computed organic matter decomposition and respiration. Moreover, similar to heterotrophic bacteria, different strains of the *Synechococcus* genus are also able to assimilate exogenous organic compounds like adenine, acetate, urea, leucine, uracil and thymidine (Martinez et al., 1989) (see Martinez & Azam, 1993 and references therein). Another important ecological implication for *Synechococcus* lies in the protein turnover because they are able to hydrolyze oligopeptides, especially in nitrogen-limited environments (Martinez & Azam, 1993).

In the present study, we investigated a picocyanobacterial mixed culture harvested from a widely monitored site in the Gulf of Trieste, which is one of the long-term research stations of the Northern Adriatic, included in the Italian Long-Term Ecological Research Network (LTER-Italy).

Cells were identified as *Synechococcus* sp. by means of an identification key (Bergey’s Manual of Systematic Bacteriology) (Castenholz, 2001), transmission electron microscopy (TEM) observations, pigment and molecular analyses. We first analyzed the ultrastructural features and biliproteins of the cells and then focused on *Synechococcus* heterotrophic-like behavior by investigating the production of exoenzymes acting on different organic compounds (proteins, polysaccharides and lipids) as well as the uptake of organic molecules (leucine and thymidine incorporation) within the culture and two reference strains from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton. We finally compared the organic carbon production via photosynthesis and via leucine incorporation, in order to evaluate the importance of the heterotrophic-like process within their carbon metabolism.

**Materials and methods**

**Preparation of culture and culture conditions**

Picocyanobacterial culture was harvested from the Gulf of Trieste (45°42′03″N–13°42′36″E) during a survey carried out in fall 2004 when *Synechococcus* generally reaches maximum abundances. Seawater collected at four depths from the surface to the bottom of the water column (max depth of 16 m) was prefiltered through a 10 μm mesh and 5 and 2 μm polycarbonate filters (Nuclepore) and then collected into an autoclaved glass flask. The prefilter was then filtered through 1 μm filters (Nuclepore) in order to minimize the heterotrophic bacteria content. The 1 μm filter content was resuspended into B medium (Agatha et al., 2004) prepared with autoclaved artificial seawater and then divided into six replicate tubes and incubated under environmental conditions: 15 °C with a 12:12 light:dark cycle under 33 μmol m−2 s−1 PAR (OSRAM L18W21-Hellweiss LUMI-LUX Cool White). After c. 4 weeks under the above described conditions enrichment cultures were maintained by periodical transfers (2 weeks) into fresh medium. The culture obtained has been named SynTS. Filtrations allowed the complete removal of all organisms from the culture except for picocyanobacteria and an exiguous amount of heterotrophic bacteria, leading to a change in the phototropic:heterotrophic bacteria ratio from 9.1·100 in seawater to < 100:1 within the culture. For comparison of exoenzymatic activities and leucine incorporation, two axenic *Synechococcus* strains, CCMP 1631 (*Synechococcus elongatus*, 248-01) and CCMP 1334 (DC2, WH7803, NEPCC549) from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP), were grown in B medium at 20 °C with a 12:12 light: dark cycle under 33 μmol m−2 s−1 PAR.

**Systematic identification, fine structure and phycobiliproteins of the cells**

The cultured cells were identified as *Synechococcus* according to the Bergey’s Manual of Systematic Bacteriology (Castenholz, 2001). For TEM (Philips EM201) observations, aliquots of the culture were either centrifuged at 5900 g for 5 min or filtered on 0.2 μm polycarbonate filters (Nuclepore). Both preparations were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.1, postfixed in 1% (w/v) osmium tetroxide in the same buffer and included in Spurr resin (Frisenda et al., 2006). *In vivo* fluorescence excitation and emission spectra (Perkin Elmer LS 50B spectrophotometer) were obtained using cultures in the logarithmic growth phase. Excitation and emission spectra were measured at fixed 652 nm λEx and 545 nm λEm maxima, respectively. Both sterile B medium and 0.2 μm filtered cultures were used as the reference, and indicated fluorescence lower than 1% with respect to the culture. Spectra were smoothed with a 5 nm moving average. Biliprotein contents were also analyzed on crude extracts (Talarico & Kosovel, 1982; Honsell et al., 1984) by spectrophotometry (Perkin-Elmer UV-VIS 554).
Cyanobacterial community structure

Culture aliquots (2 mL) were centrifuged at 20,800 g at room temperature for 10 min and then added with 175 μL of lysis buffer (400 mM NaCl, 750 mM sucrose, 20 mM EDTA, 50 mM Tris-HCl pH 9.0). DNA was extracted according to Boström et al. (2004). Briefly, cell lysis was performed by adding lysozyme (final concentration 1 mg mL⁻¹), sodium dodecyl sulfate (final concentration 1%) and proteinase K (final concentration 100 μg mL⁻¹). After the addition of 50 μg tRNA (from Saccharomyces cerevisiae, stock solution 10 mg mL⁻¹, Sigma) and 0.1 vol 3 M NaAc, DNA was precipitated with 0.6 vol isopropanol. Samples were then centrifuged and washed with 70% ethanol. The pellets were resuspended with 50 μL MQ. PCR and denaturing gradient gel electrophoresis (DGGE) were performed with cyanobacterial primers as described previously (Celussi et al., 2008). Polyclaracrylamide gel slices containing the cyanobacterial DNA band of interest were excised using a sterile scalpel and eluted in 100 μL of MilliQ water overnight at −20 °C, followed by a freeze–thaw cycle. One microliter of thawed elution was reamplified using the CYA 781R-CYA 349F (Nübel et al., 1997) primer without the GC clamp, and PCR product purified using the QIAquick PCR purification kit (Qiagen) according to the supplier’s instructions.

Samples were sequenced using ABI Prism Big Dye terminator chemistry (Applied Biosystems) using M13R primer and an ABI Prism 310 Genetic Analyzer (Applied Biosystems), according to the supplier’s instructions. Sequences were aligned to known sequences in the GenBank database using BLAST (Altschul et al., 1997). Multiple sequence alignment was performed using the CLUSTALX 1.81 program (Thompson et al., 1997); the phylogenetic relationships were inferred by the neighbor-joining method and a phylogenetic tree was constructed with the program NIVPLOT and finally edited for better graphics with CLC Free Workbench package version 4.0.1.

Synechococcus cell abundances

Culture aliquots (1 mL) were diluted 1:50 with artificial seawater and then fixed with 2% final concentration borate-buffered formalin (prefiltered through a 0.2 μm Acrodisc filter). Subsamples (5 mL) were filtered in triplicate onto 0.2-μm black-stained polycarbonate filters (Nuclepore). Filters were mounted on microscope slides, between layers of nonfluorescent immersion oil (Olympus), and counted within a few hours. Counts were made under a green (BP 480–550 nm, BA 590 nm) and a blue (BP 420–480 nm, BA 515 nm) filter set by epifluorescence microscopy (magnification, ×1000). Cells that auto-fluoresced orange were counted in at least 20 random fields and a minimum of 300 cells for each filter. Each value represents the mean of triplicate samples. Samples were processed until the coefficient of variation (CV = SD/Mean × 100) among three replicates was lower than 5%.

Heterotrophic bacteria

The presence of heterotrophic bacteria was monitored in the SynTS mixed cultures and in the CCMP 1631 and 1334 cultures during the whole experiment. Culture aliquots treated previously as described in the above section were stained for 15 min with 4’6-diamidino-2-phenylindole (DAPI, Sigma) at 1 μg mL⁻¹ final concentration (Porter & Feig, 1980) and then filtered in triplicate onto 0.2-μm black-stained polycarbonate filters (Nuclepore). Heterotrophic bacteria were counted by epifluorescence microscopy under a UV (BP 330–385 nm, BA 420 nm) filter set. In each filter, at least 20 random fields and a minimum of 300 cells were counted.

Exoenzymatic activities (EEA)

Hydrolitic exoenzyme activities were measured with fluorogenic analogs of natural substrates (Hoppe, 1993): l-leucine-4-methylcoumarinile-7-amide (MCA, aminopeptidase), methyl umbelliferyl-β-D-glucoside (MUF, β-D-glucosidase), methyl umbelliferyl-β-D-galactoside (MUF, β-D-galactosidase) and methyl umbelliferyl oleate (MUF, oleate). Hydrolysis by bacteria was measured by incubating 2.5-mL subsamples with 200 μM leucine-MCA, MUF-β-glucoside, MUF-β-galactoside and MUF-oleate substrates for 1 h at 15 °C in the dark. The fluorescence released by enzymatic cleavage of the artificial substrates was measured fluorometrically, in quadruplicate, at 380/365 nm excitation and 440/455 nm emission for MCA/MUF substrates using a Perkin Elmer LS 50B fluorometer. Aminopeptidase activity was converted to protein mobilized using the conversion factor 72 μg C μmol⁻¹.

Primary carbon production

Cells productivity was measured by the 14C technique (Steeman-Nielsen, 1952). Six aliquots (80 mL) from the SynTS mixed culture in the exponential phase of the growth curve were maintained in the dark for 30 min in order to minimize the photosynthetic activity. Replicate sample aliquots were inoculated with 222 KBq of NaH14CO3 and incubated for 2 h under culture conditions. The analysis was performed in quadruplicate against two dark bottle replicates, incubated for background measurements. At the end of incubation, 1, 2, 5 and 10-mL subsamples were acidified with a few drops of HCl 5 N for total primary production (PP) measurements. Extracellular release (ER) was measured by filtering 1, 2, 5 and 10-mL subsamples with 0.22-μm Millipore filters and then acidifying both the filter and the filtered water. 24 h after acidification, scintillation cocktail
was added to subsamples: 5 mL of Filter Count for filters and 10 mL of Ultima Gold for PP and ER. Activity was determined by a β-counter Packard Tri-Carb 300. Assimilation of carbon was calculated as described by Gargas (1975), assuming 5% isotope discrimination. Activity of the added NaH14CO3 and inorganic carbon concentration (tCO2) were determined on the basis of total alkalinity measured in the same culture.

Secondary carbon production

Secondary carbon production was measured by the incorporation of 3H-leucine (Smith & Azam, 1992) and 3H-thymidine (Tdr) (Fuhrman & Azam, 1982). Quadruplicate 1.7 mL culture aliquots and two blanks [added with 90 μL 100% trichloracetic acid (TCA)] were amended with a 20 nM radiotracer and incubated in the dark at 15 and 5 ºC for leucine and Tdr, respectively. Incubations were stopped with 100% TCA after 1 h. The extraction with 5% TCA and 80% ethanol was carried out using the microcentrifugation method (Smith & Azam, 1992). The incorporated radioactivity was determined by a β-counter (Packard Tri-Carb 300) after the addition of 1 mL scintillation cocktail (Ultima Gold MV; Packard). Incorporation of 3H-leucine was converted into carbon produced via cyanobacterial protein production according to Simon & Azam (1989), assuming a twofold isotope dilution for leucine.

Results and discussion

Fine structure, biliprotein and culture identification of cells

Within the SynTS mixed cultures harvested from the Gulf of Trieste, TEM electron microscopy highlighted the dominance of Synechococcus-like cells (Fig. 1) that divided in a single plane. Cells were cocccoid (0.8 μm) and rod shaped (from 1.0 to 1.6 μm long) with similar ultrastructural features. The external sheath(s) appeared to be poorly structured, the peptidoglycan layer between the outer and plasma membranes (Gantt, 1994) was barely visible and no periplasmic spaces were apparent. Thylakoids (thy) showed orderly parallel and concentric arrangements with peripheral location. Similar to eukaryotic red algae, interthylakoidal electron density indicated high concentrations of ‘polydisperse’ biliproteins (Talarico, 1990) that may act as a constraint on phycobilisome assembly (Talarico, 1996), depending on the light (irradiance/spectral composition) applied to the cultures (Talarico & Maranzana, 2000). In fact, only a few cells exhibited phycobilisomes (pbs) that were poorly structured and their presence is most probably due to some ‘shade effect’ of cell density in the culture. As a response to light quantity and quality, more or less structured phycobilisomes have been described for several cyanobacteria (Grossman, 2003) and eukaryotic red algae (Talarico, 1996; Talarico et al., 1998; Talarico & Maranzana, 2000), both in the field and under culture. Other features such as an electron-transparent centroplasm with DNA filaments (n), several cytoplasmic inclusions, i.e. cyanophycin granules, polyphosphate bodies (pp) and carboxisomes (c), commonly seen in cyanobacteria (Kromkamp, 1987; Lefort-Tran et al., 1988; Harano et al., 2003), were also apparent.

The in vivo fluorescence emission (Em) spectrum (Fig. 2) highlighted the presence of phycocyanobilin chromophores carried by both C-phycocyanin (652 nm Em λmax) and by inner allophycocyanins (APC, APC-B and APC680 of the chromophorylated core-membrane linker LCM) with the final emission at 680 nm λmax (Talarico & Maranzana, 2000). The in vivo fluorescence excitation spectrum showed the presence of both phycourbilin and phycoerythrobilin chromophores (Fig. 2), in agreement with previous results on crude extracts from the same culture (Frisenda et al., 2006). In fact, both absorption (Abs) and excitation (Ex) spectra revealed the presence of CU-phycoerythrin with
Fig. 2. In vivo fluorescence excitation (solid line) and emission (dashed line) spectra, measured at fixed 652 nm $\lambda_{\text{ex}}$ and 545 nm $\lambda_{\text{em}}$, respectively. The peaks corresponding to phycourobilin, phycoerythrobilin and phycocyanobilin chromophores carried by $\gamma$, $\alpha$ and $\beta$ subunits are indicated.
different pigment (phycourobilin/phycoerythrobilin) ratios have been proven to form a monophyletic group, motility being a better marker of genetic affiliation than pigment complement. These results suggest that the genetic divergence we observed (Fig. 4) may rather reflect two different physiological responses to light, i.e. chromatic ‘short term’ acclimation (Talarico, 1996; Talarico & Maranzana, 2000). These two groups would possess quite different photoacclimation capabilities, in agreement with the findings of Palenik (2001), who clearly demonstrated that some marine strains of Synechococcus under culture were able to acclimate while others were not, despite similar phycourobilin and phycoerythrobilin spectral signatures. The hypothesis that these two lineages reflect two different physiological behaviors, possibly discriminating light (irradiance/spectral composition) ‘adapters’ and ‘nonadapters’, is further supported by the recent findings of Ahlgren & Rocap (2006), who isolated two new marine Synechococcus ecotypes with distinctive light (and nitrogen) physiologies under culture, thus proving that chromatic ‘short term’ adaptation is more widely distributed among Synechococcus strains than known previously. This underlines the need for further exploration of the physiological differences that allow for the coexistence of ecotypes fulfilling the same ecological niche. Additional insights into the adaptive mechanisms to light and other environmental factors would contribute to a new understanding of ecotype differentiation in the marine Synechococcus lineage (Ahlgren & Rocap, 2006), both on evolutionary and on physiological time scales. It is foreseeable that an increased number of genome sequences of cultured isolates, when available, will allow the comparison of genomic and physiological data that is crucial for understanding the ecological role (Hess, 2004) of these so widely distributed picocyanobacteria.

**Synechococcus** heterotrophic-like behavior

In Table 1, the range of variation of cells’ abundance, aminopeptidase activity (AMA), protein carbon mobilization (C mob), Leucine incorporation and percentage of mobilized carbon utilization (C mob utilization) measured during the growth curve of the SynTS mixed culture and of the two axenic strains (CCMP 1334 and CCMP 1631) are reported. In Fig. 5, the trends of the above-mentioned parameters for the SynTS mixed culture are reported. During the whole study, the CCMP 1631 and 1334 cultures remained axenic while the phototrophic : heterotrophic bacteria ratio within the SynTS mixed culture ranged between 40 : 1 and 8 : 1 (Fig. 5), which means more than three orders of magnitude higher than the seawater one. The EEA were measured in order to characterize the carbon-containing substrates degradable by *Synechococcus* cultured strains: proteins (AMA), polysaccharides (β-glucosidase and β-galactosidase activities) and lipids (lipase activity). Among the four EEA investigated, only aminopeptidase showed high hydrolytic activity both for SynTS culture and for CCMP strains (Table 1). As described previously (Paoli et al., 2005), high activities of the enzyme alkaline phosphatase were also found within the same cultures. The incorporation of macromolecules within cultures was positive for leucine but not for Tdr. Martinez & Azam (1993) also found high AMA within different Synechococcus strains, and thymidine incorporation was found for *Synechococcus curtus*.
Table 1. Maxima and minima of cell abundance, aminopeptidase activity (AMA), protein carbon mobilization (C mob), Leu incorporation (Leu), and percentage of mobilized carbon utilization (C mob utilization) measured during the growth curve of a Synechococcus mixed culture harvested from the Gulf of Trieste (SynTS) and two axenic cultures (CCMP 1334 and CCMP 1631) from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP).

<table>
<thead>
<tr>
<th></th>
<th>SynTS</th>
<th>CCMP 1334</th>
<th>CCMP 1631</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>Abundance</td>
<td>$10^8$ cells L$^{-1}$</td>
<td>$39.4 \pm 2.9$</td>
<td>$1827 \pm 76$</td>
</tr>
<tr>
<td>AMA</td>
<td>$\mu$M h$^{-1}$</td>
<td>$0.2 \pm 0.02$</td>
<td>$15.7 \pm 0.9$</td>
</tr>
<tr>
<td>C mob</td>
<td>$\mu$g C L$^{-1}$ h$^{-1}$</td>
<td>$14.8 \pm 2.2$</td>
<td>$1127 \pm 64$</td>
</tr>
<tr>
<td>Leu</td>
<td>$\mu$g C L$^{-1}$ h$^{-1}$</td>
<td>$1.7 \pm 0.06$</td>
<td>$18.5 \pm 1.3$</td>
</tr>
<tr>
<td>C mob utilization</td>
<td>%</td>
<td>$1.6 \pm 0.01$</td>
<td>$11.6 \pm 0.1$</td>
</tr>
</tbody>
</table>

Each value represents the mean of triplicate (for cells abundance) and quadruplicate (for others) sample analysis ± SD. The standard propagated error is indicated for the C mob utilization.

(Martinez et al., 1989) but not for the WH7803 strain (Cuhel & Waterbury, 1984). AMA paralleled the Synechococcus growth curves, and leucine incorporation rates increased strongly during the lag phase and then weakly and progressively during the whole exponential growth until the beginning of the senescent state of the cultures (see Fig. 5 for SynTS). AMA and leucine incorporation values are extremely high when compared with the seawater of the North Adriatic as well as the activities detected in mucilage aggregates (see Del Negro et al., 2005 and references therein). AMA and leucine incorporation rates within SynTS culture were in the range of those found for the CCMP 1631 and CCMP 1334 strains (Table 1). The protein C potentially mobilized by AMA was particularly high when compared with the leucine incorporation (Table 1), resulting in low percentages of newly mobilized C utilization. Nevertheless, the SynTS culture showed the highest percentage of C utilization (up to 11.6%) among the investigated cultures, with the highest values in the lag phase decreasing toward the end of the growth curve (Fig. 5). We hypothesize that the higher percentage of C taken up with respect to the C mobilized in the lag phase would sustain the cell division processes characterizing the upcoming exponential phase. Because the aminopeptidase activity and leucine incorporation rates within the SynTS mixed culture were in the range of those found for CCMP 1631 and CCMP 1334, and by considering that the phototrophic:heterotrophic bacteria ratio within SynTS culture was always more than three orders of magnitude higher than the seawater one, we concluded that in the latter culture both activities could be primarily ascribed to Synechococcus cells and the heterotrophic bacterial contribution is negligible. The presence of high aminopeptidase activity and secondary carbon production within the mixed cultures confirms the role of
Synechococcus in C remineralization, particularly through the degradation/utilization of proteins and not of sugars or lipids. Notwithstanding leucine incorporation is generally considered to be an important mode of carbon utilization for marine organisms; this process could also represent an important strategy for nitrogen recovery for Synechococcus. As a result of their strong N dependence (Collier et al., 1999), most of the marine cyanobacteria developed some strategies that are alternative to inorganic N absorption like N₂ fixation, exoenzymatic degradation and direct uptake of low-molecular-weight organic compounds rich in N. A study on Synechococcus revealed that its element assimilation ratio (C:N:P:S = 95:16:3:1) is quite high for N (Cuhel & Waterbury, 1984); thus, leucine and other amino acids would represent an important source of organic nitrogen. The high leucine incorporation rate may also represent an energetically less expensive way for ‘constructing’ biliproteins, given their massive presence, as discussed previously. It is reasonable to think that biliproteins would represent an important inner N source in cases of N limitation in the surrounding environment.

Measurements of the primary carbon production (PP) were performed within SynTS culture during the exponential phase of the growth curve. Synechococcus cells and heterotrophic bacterial abundances were c. 5.4 x 10^10 and 1.1 x 10^9 cells L⁻¹, respectively. The PP analyses showed similar results by considering different aliquots of the same replicates (1, 2, 5 and 10 mL). A total production of 34.6 ± 0.9 µg C L⁻¹ h⁻¹ with no detectable extracellular release (Fig. 6) was observed. Thus, the inorganic carbon fixed by photosynthesis was completely transformed in biomass and not released by exudation processes or the exuded carbon, if any, was immediately consumed by the heterotrophic bacteria. The comparison of the organic carbon produced photosynthetically with the organic carbon incorporated as leucine (11.1 ± 0.3 µg C L⁻¹ h⁻¹) yielded an c. 3:1 ratio (Fig. 6).

According to these results, a clear photoheterotrophic behavior characterizes the Synechococcus cells harvested for marine organisms; this process could also represent an important strategy for nitrogen recovery for Synechococcus.
from the Gulf of Trieste and this might represent a key to the successful large-scale distribution of this genus within this area as well as over the entire Adriatic and Mediterranean sea. In addition to the important ecological advantages given by their small dimension (i.e. a reduced sedimentation rate, a more efficient light energy absorption, together with a more efficient inorganic nutrients’ adsorption) (Fogg, 1995), their ability to adapt chromatically to underwater light fields represents an important mechanism for their competitive dominance over planktonic microalgae. Moreover, the capacity to regenerate N and P inorganic nutrients (Martinez & Azam, 1993; Paoli et al., 2005) and to utilize organic compounds taken up from the surrounding water (Cuhel & Waterbury, 1984; Martinez et al., 1989) allows Synechococcus to live in different marine environments, from oligotrophic to highly productive ones. In the Gulf of Trieste, which is characterized by a generally low trophic state (Fonda Umani et al., 2007) that could rapidly turn into a very high state due to the impulsive nature of the local rivers’ outflow, Synechococcus cells are always abundant during the whole year (Paoli & Del Negro, 2006). They might be adapted to directing their metabolism to remineralization rather than production processes in relation to the temporary trophic state of the basin. The ability of Synechococcus to adapt to an annual (but also on a short time-scale of days) (Paoli et al., 2006) wide variation in hydrological and chemical-physical factors (Cushman-Roisin et al., 2001) may help to explain the significant increase in their abundances over the last decades in the Gulf of Trieste (Paoli & Del Negro, 2006), whereas a general decrease in microphytoplankton abundance (and particularly diatoms) was observed (Fonda Umani et al., 2004). The Synechococcus role in the degradation of the organic matter as well as in the regenerated production processes might place them in competition with the heterotrophic bacteria and this might justify their similar, both seasonal and multiyear, patterns of distribution (Paoli & Del Negro, 2006), and conversely, photosynthetic cyanobacteria can utilize key nutrients and organic compounds heterotrophically.

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