Iron-Limited Biomass Yields of Marine Phytoplankton Clones

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철의 첨가량 제한에 따른 해양 식물플랑크톤 단종배양체의 생체량증가

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The final biomass yields of 16 marine phytoplankton clones were measured in media with different levels of iron and phosphorus concentrations. The biomass yields of oceanic clones were either only slightly limited or not limited by iron, and those of coastal clones were severely limited by iron in all phylogenetic groups other than cyanobacteria. By contrast oceanic cyanobacterial clones as well as coastal clones required higher iron concentrations: minimum concentrations of iron addition for detectable growth of the *Synechococcus* species were estimated to fall between 10^{-9} and 10^{-8} M. Not only the habitat differences (oceanic-coastal trends) but also the phylogenetic differences of the oceanic phytoplankton species in the response to the iron enrichments deserve very careful attention before 'ocean iron fertilization'.

해양 식물플랑크톤의 단종배양체 16종류를 대상으로 하여, 배양액 중의 철 및 인 농도차이에 따른 최종 생체량증가 정도를 측정하였다. 시아노세균을 제외한 나머지 모든 분류군 가운데, 철은 원양성 단종배양체의 최종 생체량 증가 정도를 약간 제한하거나 전혀 제한하지 않으며, 연안성 단종배양체의 최종 생체량 증가 정도는 심하게 제한한다. 대조적으로, 시아노세균의 연안성 단종배양체와 원양성 단종배양체 모두 고농도 철의 요구성을 나타낸다: *Synechococcus* 종류들이 측정한계 이상의 성장치를 나타내기 위한 철의 최저농도는 10^{-9}과 10^{-8} M 사이에 있다. 소위 '철을 이용한 대양의 비옥화' 계획을 실행하기 전에, 철의 증가에 따른 원양성 식물플랑크톤 종들의 반응이 서식환경에 따라(원양역-연안역 간) 그리고 분류군에 따라 어떠한 차이를 나타내는지 세심한 주의를 기울여야 한다.

INTRODUCTION

H. H. Gran firstly suggested the possibility of iron-limited growth of marine phytoplankton species in some parts of the oceans in early 1930s (Mills, 1989). The data on the oceanic distribution of iron are very few because of its low concentration in oceanic waters and aerial iron contamination on ships and in laboratories (Burton and Sta-

tham, 1990). Therefore, the quantitative studies on the iron limitation of oceanic phytoplankton drew attention only recently (Sunda et al., 1991; De Baar et al., 1990; Martin et al., 1989; Brand et al., 1983). Oceanic diatoms (Sunda et al., 1991; Brand et al., 1983) and oceanic coccolithophores (Brand et al., 1983) were reported to require lower iron concentrations for their growth than the coastal species. Addition of nmol amounts of dissolved iron to

the Gulf of Alaska samples resulted in the rapid growth of the phytoplankters in the samples, and the amounts of the chlorophyll in the phytoplankton increased in proportion to the iron added (Martin and Fitzwater, 1988). Aerial dust input to the ocean has been hypothesized to increase oceanic primary productivity, lowering the atmospheric CO_2 levels in gological time scales (Martin et al., 1989). Thus, the addition of iron to the Southern Ocean to remove atmospheric CO_2 for global environmental preservation was suggested by Martin et al. (1990), and criticized severely by Banse (Banse 1990; Martin et al., 1990; Banse, 1991). Banse (1991) pointed out the incompleteness in our knowledge on the marine phytolankton ecology associated with the iron limitation, e.g., the role of the grazers, species succession after the iron addition, and long-term (longer than several days) response

of the photosynthetic organisms to the iron enrichment.

Here we present the results on the final biomass yields of the 16 coastal and oceanic phytoplankton clones following iron addition to the culture media. Phylogenetic differences among the clones as well as the differences in habitats (coastal or oceanic) were examined in reference to their levels of biomass yields.

MATERIALS AND METHODS

The experimental cultures for the present study (Table 1) were established by single cell isolation before enrichment (Guillard, 1973) or obtained from Provasoli-Guillard Center for Culture of Marine Phytoplankton (Andersen et al., 1991), although sterile techniques were applied to the entire

Table 1. The origin of the clonal cultures.

clone	species name	location	year	isolator
Coccolithophores				
i) oceanic				
1387	Emiliania huxleyi	23°48.8′N 89°45.7′W	1980	L. Brand
451B	Emiliania huxleyi	Oslo fjord, Norway	1959	E. Paasche
2221	Gephyrocapsa oceanica	08°53.7′N 79°31.7′W	1983	L. Brand
2390	Umbilicosphaera sibogae	24°19.5′N 82°12.2′W	1990	L. Brand
ii) coastal				
Copel	Coccolithus pelagicus			R. Guillard
Cocco2	Pleurochrysis carterae	Woods Hole, MA, USA	1958	I. Pintner
Se62	Syracosphaera elongata			M. Droop
Dinoflagellate				
i) oceanic				
Wt8	Gymnodinium simplex	09°48.5′N 89°14.5′W	1958	A. Dodson
ii) coastal				
Amphi	Amphidinium carterae	Great Pond, Falmouth, MA, USA	1954	L. Guillard
Exuv	Prorocentrum minimum	Great South Bay, LI, USA	1956	I. Pintner
other Eucaryotes				
i) oceanic				
MC1	Mantoniella sp.	North Pacific Central Gyre	1973	R. Lewin
2167	Micromonas sp.	05°08.1′S 81°43.1′W	1983	L. Brand
ii) coastal				
Olisth	Heterosigma akashiwo	Long Island Sound, NY, USA	1952	R. Conover
Cyanobacteria				
i) oceain				
DC2	Synechococcus sp.	33°44.9′N 67°29.8′W	1978	L. Brand
2357	Synechococcus sp.	N. Tongue of the Ocean, Atlantic	1984	L. Brand
ii) coastal				
SYN	Synechococcus bacillaris	Milford, CT, USA	1957	R. Guillard

Table 2. Combinations of iron and phosphrus concentrations ($-\log M$) added to the culture media.

species	Medium1	Medium2	Medium3	Medium4	Medium5	Medium6	Medium7
iron	no	10	9	8	7	6	6
phosphorus	5	5	5	5	5	5	5.301

experiments, most of the cultures were probably not axenic. Basal seawater for the culture media was collected from the Northern Tongue of the Ocean, Bahamas. The seawater and individual nutrient stock solutions were all tyndallized separately in teflon bottles. The 37% salinity seawater was enriched with 10^{-3} M $NaNO_3$, 10^{-5} M NH_4Cl, 10^{-4} M Na_2SiO_3, 10^{-7} M EDTA, 10^{-8} M $ZnSO_4$, 10^{-8} M $MnSO_4$, 10^{-9} M $CuSO_4$, 10^{-9} M $CoSO_4$, 10^{-8} M biotin, 10^{-8} M thiamine, 10^{-9} M vitamin B_{12}, either 5×10^{-6} or 10^{-5} M NaH_2PO_4 and various concentrations of Fe-EDTA (Table 2). For the experiments 25×100 mm polycarbonate test tubes with screw caps were used after a cleaning procedure. The procedure includes cleaning with detergent and repeated rinses and soaks of dilute HCl and deionized water (18 megaohm), and final rinsing with the tyndallized seawater. The experimental culture were incubated at 21-23°C, and a light intensity of 65 $\mu E/m^2/sec$ was maintained with a 12:12 photoperiod. New batch cultures were established sequentially by transferring an inoculum from each batch culture at its peak abundance. To monitor the final biomass yield in different culture media, *in vivo* fluorescence was measured with a Turner 10-000R fluorometer.

RESULTS

The final biomass yields measured in each nutrient regime (Table 2) are illustrated in Fig. 1, 2, 3, 4 and 5. In the iron limited species (Fig. 2, 4 and 5) 10^{-10} M iron additions resulted in negligible increase in biomass yields, and 10^{-9} M iron resulted in evident increase. This fact implies that the background iron concentration in the basal
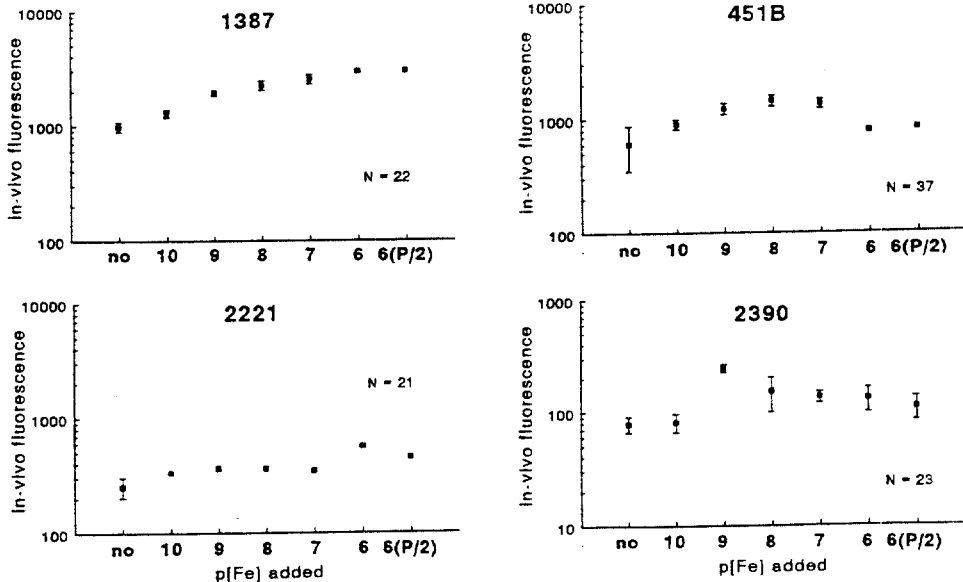

Fig. 1. Final biomass yields of oceanic coccolithophores (1387-*Emiliania huxleyi*, 451B-*Emiliania huxleyi*, 2221-*Gephyrocapsa oceanica*, 2390-*Umbilicosphaera sibogae*) by iron addition. The mean and SEM (standard error of mean) of replicate analyses are shown. N is the total number of the measurements of the final biomass yields. Phosphorus concentrations were as described in Table 2.
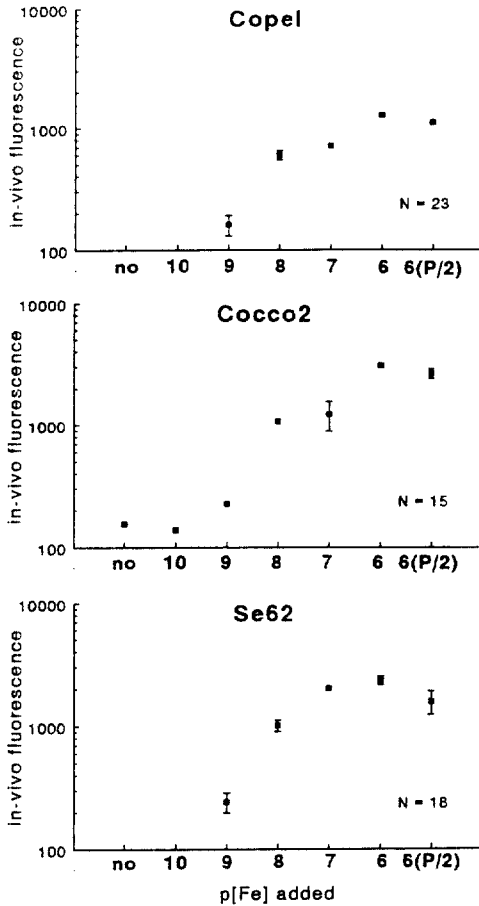
Fig. 2. Final biomass yields of coastal coccolithophores (Copel-*Coccolithus pelagicus*, Cocco2-*Pleurochrysis carterae*, Se62-*Syracosphaera elongata*, by iron addition. The mean and SEM (standard error of mean) of replicate analyses are shown. N is the total number of the measurements of the final biomass yields. Phosphorus concentrations were as described in Table 2.
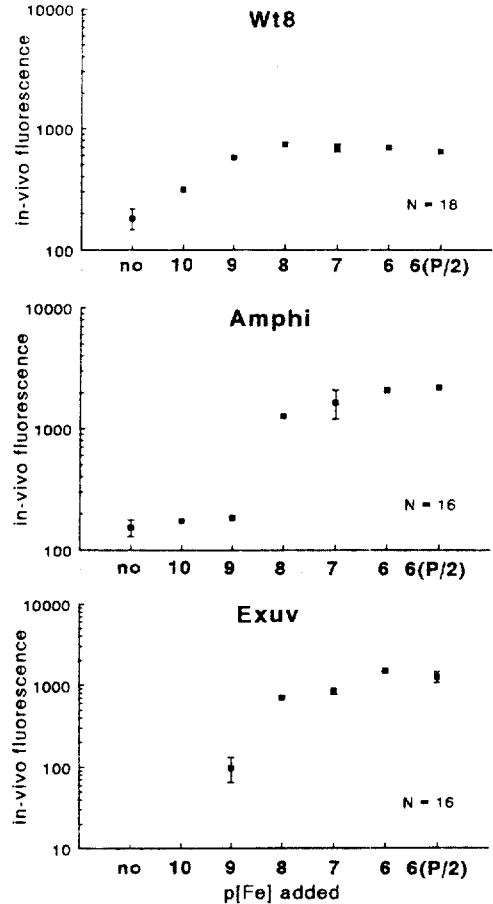
Fig. 3. Final biomass yields of an oceanic clone (Wt8-*Gymnodinium simplex*) and two coastal clones (Amphi-*Amphidinium carterae*, Exuv-*Prorocentrum minimum*) of dinoflagellates by iron eddition. The mean and SEM (standard error of mean) of replicate analyses are shown. N is the total number of the measurements of the final biomass yields. Phosphorus concentrations were as described in Table 2.

seawater for the culture media may be around 10^{-10} M.

Coccolithophores

Figure 1 shows the biomass yields of oceanic coccolithophores, and Figure 2 shows the yields by coastal coccolithophores. In all the coastal clones the final biomass yields were limited sharply by low iron concentrations, and two (Copel, *Coccolithus*

pelagicus, and Se62. *Syracosphaera elongata*) of the three coastal clones showed no growth in media with 10^{-10} M iron addition (Fig. 2). By contrast none of the oceanic species were limited by iron, and almost the same levels of biomass yield were achieved in all the iron concentrations tested for each clone (Fig. 1). In an oceanic species, *Emiliania huxleyi*, only slight increase in biomass yields at higher iron concentration is detected (Fig. 1), which is insignificant compared with the varia-
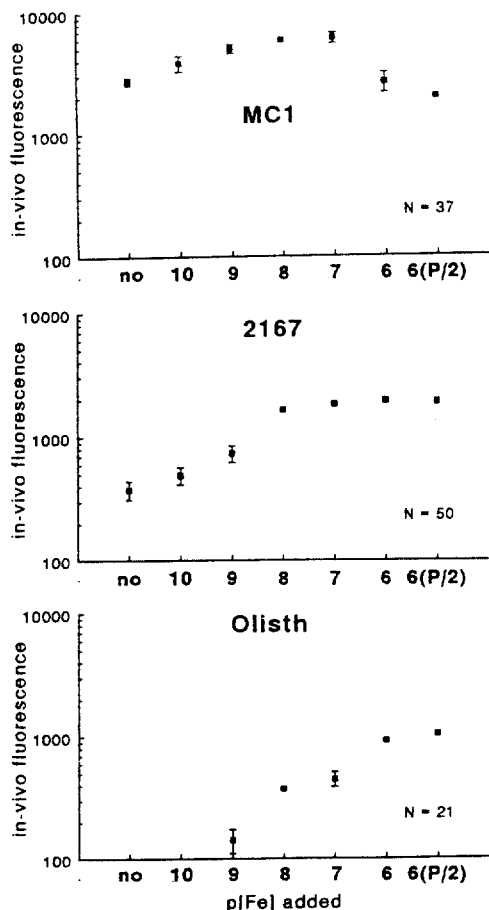
Fig. 4. Final biomass yields of two oceanic prasinophytes (MC1-*Mantoniella* sp., 2167-*Micromonas* sp.) and a coastal raphydophyte (Olishth-*Heterosigma akashiwo*, by iron addition. The mean and SEM (standard error of mean) of replicate analyses are shown. N is the total number of the measurements of the final biomass yields. Phosphorus concentrations were as described in Table 2.
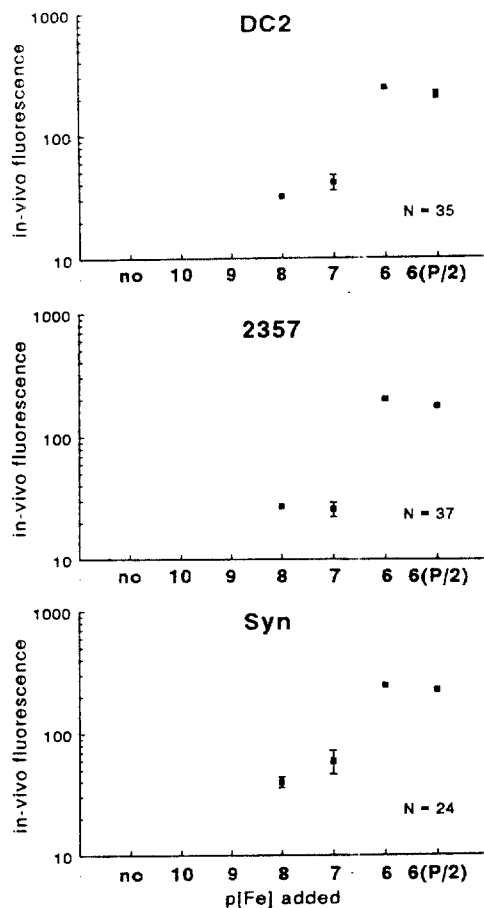
Fig. 5. Final biomass yields of two oceanic clones (DC2-*Synechococcus* sp., 2357-*Synechococcus* sp.) and a coastal clone (SYN-*Synechococcus bacillaris*) of unicellular cyanobacteria by iron addition. The mean and SEM (standard error of mean) of replicate analyses are shown. N is the total number of the measurements of the final biomass yiedls. Phosphorus concentrations were as described in Table 2.

tions in the coastal species (Fig. 2).

Dinoflagellates

The biomass yields of the two coastal dinoflagellate species (*Amphidinium carterae* and *Prorocentrum minimum*) were severely limited by iron (Fig. 3). However, an oceanic species, *Gymnodinium simplex*, showed slight decrease of the biomass yields at the added iron concentration less than 10^{-10} M. The degree of the limitation of biomass yields

by iron in *Gymnodinium simplex* is considerably weaker than those in *Amphidinium carterae* or *Prorocentrum miniumum*.

Other eucaryotes

A raphydophyte, *Heterosigma akashiwo*, showed no growth at the iron addition of 10^{-10} M. and its biomass yields were sharply limited by iron (Fig. 5). The final biomass yields of the two ocea-

nic pransinophytes (Fig. 5) were either not limited (*Mantoniella* sp.) or only slightly limited (*Micromonas* sp.) by iron.

Unicellular Cyanobacteria

The biomass yields of a coastal unicellular cyanobacterium, *Synechococcus bacillaris*, were severely limited by iron (Fig. 5), which is the same trend as observed in other coastal clones mentioned above (Figs. 2, 3 and 4). Presenting a striking contrast to the oceanic clones in other phylogenetic groups (Figs. 1, 3 and 4), the two oceanic *Synechococcus* clones, DC2 and 2357, also showed a very sharp limitation of their biomass yields by iron (Fig. 5).

DISCUSSION

Brand (1991) examined the minimum iron requirements in 22 marine phytoplankton clones, and found out their minimum Fe : P molar ratio quotas ranging from 10^{-14} to 10^{-4} or less. Thus the highest Fe : P ratio of 2×10^{-10} applied to the media in the present experiment (Table 2) exclude any possibility of limitation of biomass yield by phosphorus.

The final biomass yields of eucaryotes by iron additions showed a habitat related pattern i.e., oceanic-coastal differences (Figs. 1, 2, 3 and 4). In other words the coastal eucaryotes (Figs. 2, 3, and 4) were severely limited by low iron concentrations while oceanic eucaryotes (Figs. 1, 3, and 4) were either slightly limited (1387 *Emiliania huxleyi*, Wt8 *Gymnodinium simplex*, and 2167 *Micromonas* sp.) or not limited. Minimum cellular Fe : C ratios supporting the maximum specific growth rates were measured in an oceanic diatom (*Thalassiosira oceanica*, clone 13-1) and an estuarine diatom (*T. pseudonana*, clone 3H) using ^{55}Fe and ^{14}C isotopes (Sunda et al., 1991). For the same specific growth rate of 1.0 per day the oceanic diatom required a cellular Fe : C ratio of 2 μmol:mol which is one fourth of the amount for the estuarine diatom (Sunda et al., 1991). Brand et al. (1983) reported the similar habitat related pattern (oceanic-ne-

ritic differences) in the limitation of marine phytoplankton reproductives rates by iron. The 21 clones of marine phytoplankton in the study (Brand et al., 1983) includes 13 diatoms, 6 coccolithophores, an oceanic dinoflagellate, and a coastal cyanobacterium.

Two oceanic *Synechococcus* clones (DC2 and 2357) as well as a coastal *Synechococcus* species (SYN) all were severely limited by iron (Fig. 5). It is very interesting to learn that there exists not only a habitat related pattern (oceanic-coastal difference) but also a phylogenetic trend in the phytoplankton biomass yields by added iron: cyanobacterial clones had different respones to the added iron from the clones in othere phylogenetic groups (Figs. 1, 2, 3, 4 and 5). The original oxygenic procaryote with plastocyanin might evolve about one billion years ago (Tappan and Loeblich, 1973) when the O_2 partial pressure in the air was around 10^{-30} atmospheric pressure (Ochiai, 1978). Therefore the seawater at that time might be much more reductive than the present seawater (Ochiai, 1978), which would drive the evolution of the early procaryotic phytoplankters to grow best in the seawater with high concentration of available iron (10^{-3} M).

Offshore surface waters of Antarctic and Gulf of Alaska were infertile without supplemental iron from the atmosphere, continental margin, or melting sea ice (Martin et al., 1989; Martin et al., 1990). Thus, the 'ocean iron fertilization' to enhance phytoplankton production was suggested as a feasible method of atmospheric CO_2 removal (Martin et al., 1990). Our data on the final biomass yields by added iron indicates that the cyanobacterial cells should outcompete the eucaryotic phytoplankters following the iron fertilization of the oceanic waters. Moreover, N_2-fixing cyanobacteria would be the best candidates among the outcompeting procaryotic phytoplankters, and the possible complex interactions in the oceanic ecosystem deserve very careful attention long before the execution of oceanic iron fertilization.

ACKNOWLEDGEMENTS

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