スーパー珪藻
Chaetoceros salsugineum

・新種ではなく、世界各地の内湾や河口域に出現する
・高野（1983）により初めて論文記載される
・細胞サイズ：3～6ミクロン

様々な重要性と可能性

【自然生態系における重要性】

一次生産者として

干潟域の高い生物生産を支える重要種？

スーパー珪藻の普遍性

高松干潟の株だけが特別なのか？他海域の株の増殖性は？

【学術的な注目点】

光合成のシステム

明反応系に特異なシステムが存在？同様に炭素の固定は？

細胞複製のメカニズム

これだけ高速でどうやって細胞を複製しているのか？

【有効利用の可能性】

水産生物の餌料として

二枚貝や動物プランクトンの餌生物として利用可能

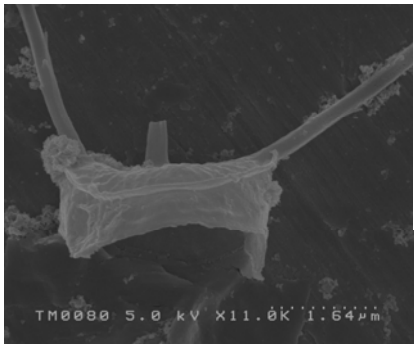
有用資源としての活用

CO2の吸収源
リン資源の吸収剤
バイオ燃料

遺伝子資源として

高速成長に関与する遺伝子の生物工学的利用

C. salsugineum
（光学顕微鏡写真）



（走査型電子
顕微鏡写真）

以下、スーパー珪藻の分裂増殖

参 考 資 料 1

■研究の概要

これまでに報告されてきた植物プランクトンの増殖速度（成長速度）は、一般的には 1 日に 1～3 分裂程度で、特に成長能力が高い一部の植物プランクトンでも 1 日にせいぜい 4 分裂程度です。1 日に 1 分裂するということは、今日 1 細胞だったものが翌日には 2 細胞になるということで、1 日に 4 分裂すれば、翌日には 16 細胞に増えるということを意味します。

我々の研究グループは、香川県高松市内の河口干潟域において、これまでに知られている成長速度とは桁違いに大きな成長速度を持った植物プランクトン（珪藻類：以下、スーパー珪藻）を発見し、飼育株を保有することに成功しました。スーパー珪藻は夏季の沿岸環境を模擬した高水温と高照度の環境下で 1 日あたり 10 分裂程度のスピードで増殖します。1 日に 10 分裂するということは、24 時間で 1,000 倍以上に増えることを意味し、現在知られている光合成生物の中で世界最速の成長スピードを持った種類と思われます。

今回、Journal of Phycology 誌には研究速報としてその成長スピードを全世界に発表しましたが、スーパー珪藻の増殖能力から、今後は以下の可能性に注目し、研究を発展させたいと考えています。

（1）干潟は熱帯雨林に次ぐ高い生物生産性を有しているが、スーパー珪藻は食物連鎖の出発点として上位生物群集の生物生産に大きく貢献している。
【自然生態系における重要種】

（2）水産生物（二枚貝や動物プランクトン）の餌料として迅速な供給が出来る。国内はもとより、熱帯・亜熱帯地域ではさらに効果的と考えられる。
【水産業界への貢献】

（3）極めて高い光合成活性を持つことから CO_2 の吸収源として、あるいは肥料物質の吸収剤として利用できる。
【CO_2 削減、有用資源捕集への利用】

（4）天然遺伝子資源として重要であることに加え、遺伝子工学的手法を応用することでバイオ燃料をはじめとした有用物質を効率よく生産できる。
【生物工学分野、エネルギー問題への利用】

＜掲載論文＞

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NOTE

EXTREMELY HIGH GROWTH RATE OF THE SMALL DIATOM CHAETOCEROS SALSUGINEUM ISOLATED FROM AN ESTUARY IN THE EASTERN SETO INLAND SEA, JAPAN[1]

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Small single-celled Chaetoceros sp. are often widely distributed, but frequently overlooked. An estuarine diatom with an extremely high growth potential under optimal conditions was isolated from the Shinkawa-Kasugagawa estuary in the eastern part of the Seto Inland Sea, western Japan. It was identified as Chaetoceros salsugineum based on morphological observations. This strain had a specific growth rate of 0.54 h^{-1} at 30°C under 700 l mol · m^{-2} · s^{-1} (about 30% of natural maximal summer light) with a 14:10 L:D cycle; there was little growth in the dark. However, under continuous light it grew at only 0.35 h^{-1} or a daily specific growth rate of 8.4 d^{-1}. In addition, cell density, chlorophyll a, and particulate organic carbon concentrations increased by about 1000 times in 24 h at 30°C under 700 l mol · m^{-2} · s^{-1} with a 14:10 L:D cycle, showing a growth rate of close to 7 d^{-1}. This very rapid growth rate may be the result of adaptation to this estuarine environment with high light and temperature. Thus, C. salsugineum can be an important primary producer in this estuary in summer and also an important organism for further physiological and genetic research.

Key index words: estuarine diatom; Chaetoceros; growth rate; high light; high temperature

There are many reports of the growth rate of cultured marine phytoplankton species isolated from coastal or oceanic habitats that have been grown under various temperatures and light intensities (Eppley 1977, Furnas 1990). Phytoplankton species growing over a temperature range 2–41°C, and a light intensity range 4–420 l mol · m^{-2} · s^{-1} with a light:dark cycle, have specific growth rates ranging from 0.14 to 3.0 d^{-1} (converted from log $_2$ to ln units per day). Higher growth rates for marine diatoms, such as 4.2 d^{-1} for Chaetoceros gracilis (Thomas 1966), or close to 3.5 d^{-1} for C. calcitrans (Thompson et al. 1992), have also been reported, although these rates were obtained under continuous light.

Phytoplankton growth is often concentrated in the subsurface layer where the light intensity is not so high and high temperatures around 30°C rarely occur in northern middle latitudes even in coastal areas. Most of the previous culture studies were carried out at relatively low temperatures and light intensities. Therefore, there is a lack of studies on phytoplankton growth rates at both high temperatures and light intensity that are typical of some shallow estuarine ecosystems in summer. Further studies using estuarine phytoplankton species and higher temperatures and irradiances are required to determine the potential growth rates of phytoplankton growing in this special habitat.

We isolated a small diatom, Chaetoceros salsugineum Takano, which has an extremely high growth rate from the Shinkawa-Kasugagawa estuary located near Takamatsu city in the eastern part of the Seto Inland Sea, Japan. This eutrophic estuary comprises a

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relatively wide tidal flat (~80 ha) with a mean tidal range of <2 m (Magni and Montani 1997). In 2005 at high tide, the annual range of water temperatures and salinity were 5.4–34.4°C and 7.2–29.4, respectively (Ichimi et al. 2008a). In this study, we report the extremely high growth potential of this species when it was grown under both high temperature and irradiance.

The dominant phytoplankter, a very small Chaetoceros, was isolated by micro-pipetting a single cell from seawater collected at Stn B (34° 21.054′ N, 134° 04.881′ W: Ichimi et al. 2008b) in August 2006. The clonal culture (strain C.sal.SK-0608) was maintained in modified ES culture medium. The medium was based on natural seawater (salinity ~31 psu) collected from the Seto Inland Sea. It was enriched with nutrients as follows: 1.4 mM $NaNO_3$, 28.7 l M K_2HPO_4, 88.0 l M $Na_2SiO{\cdot}9H_2O$ (N:Si:P = 49:3:1), trace metals, vitamins, and Tris was added with pH adjustment to ~8.2 (Okaichi et al. 1983). Cultured cells were collected during the log phase growth to identify the species. After fixation with 1% glutaraldehyde and removal of organic matter on the cell surface with an alkali solution, the fine structure of the cells was observed using a scanning electron microscope (SEM) and transmission electron microscope (TEM). Chain-forming cells with 3–4 cells or sometimes >6 cells were also observed frequently during mid-log phase growth, but many solitary cells were observed especially in late log phase growth. The cell diameter was ~3–6 l m.

The characteristic fine structure of the cells observed under SEM and TEM was the same as Chaetoceros salsugineum Takano (Takano 1983, Orlova and Selina 1993). There was a central process on the terminal valve (Fig. 1a) and on some intercalary sibling valves (Fig. 1b). The tube of the process was flattened (Fig. 1c). The seta showed twisted longitudinal costae (Fig. 1, c and d) with spinules along the seta (Fig. 1e).

The C. salsugineum was grown at 30°C under 700 l mol · $m^{)2}$ · $s^{)1}$ (using white fluorescent lamps) with a 14:10 L:D cycle. To investigate the correlation between the increase in cell density and in vivo chlorophyll fluorescence, 10 mL of culture in late log phase growth was inoculated into 1 L of modified ES culture medium and 10 sub-samples were obtained during the 14 h light period. In vivo chlorophyll fluorescence was monitored using a fluorometer (model 10-AU; Turner Designs, Sunnyvale, CA, USA). Cell densities were determined microscopically. A good correlation between cell density and in vivo chlorophyll fluorescence was obtained as follows (Fig. 2);

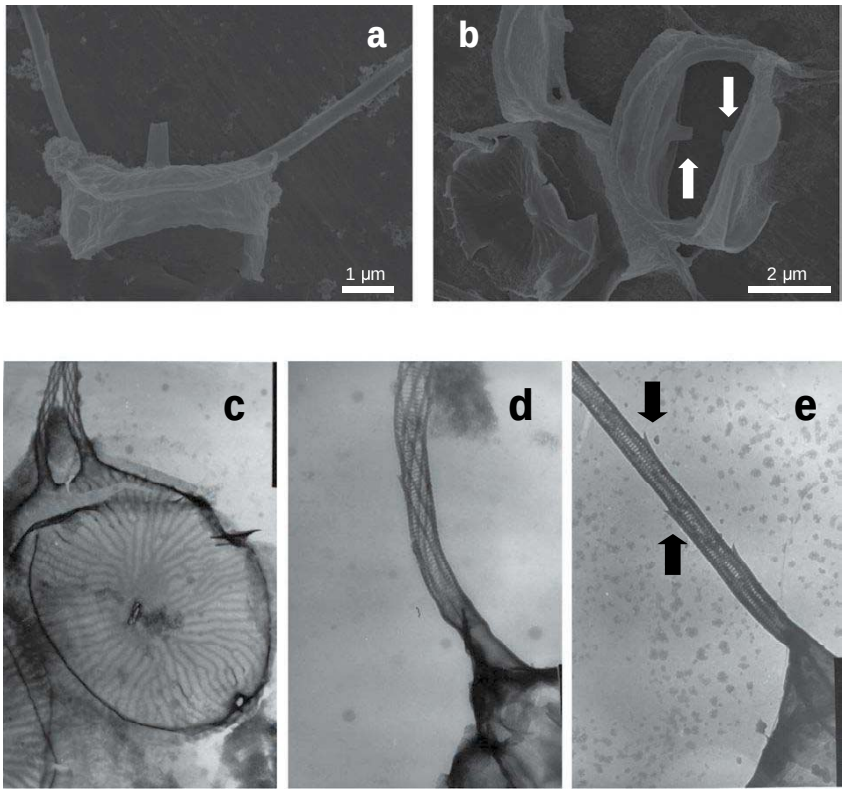

FIG. 1. Scanning electron microscope (SEM) and transmission electron microscope (TEM) of Chaetoceros salsugineum. (a) SEM – A terminal valve with a central process, (b) SEM – Central processes on intercalary sibling valves (arrows), (c) TEM – A terminal valve with a central process, (d) TEM – Seta showing twisted longitudinal costae, (e) TEM – Spinules along the seta (arrows).

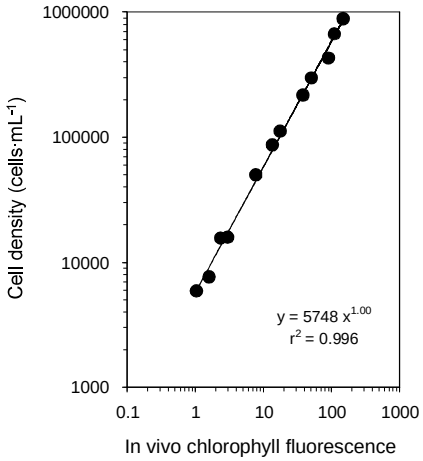

FIG. 2. Correlation between cell density and in vivo chlorophyll fluorescence for Chaetoceros salsugineum grown during the light period.

$$\text{Cell density}(\text{cells}\cdot\text{mL}^{-1}) = 5748\cdot(\text{Chl fluor})^{1.00} \qquad r^2 = 0.996 \qquad (1)$$

To determine the growth characteristics of C. salsugineum, three experiments were carried out at 30°C under 700 l mol $\cdot$ m$^{-2}\cdot$ s^{-1} to determine the growth rate in the light and dark periods of the L:D cycle (Exp. 1 & 2, respectively) and during continuous light (Exp. 3). After the cultures were acclimated for two transfers, the cells in late log phase growth were inoculated into triplicate glass tubes with 30 mL of modified ES culture medium. All culture experiments were stirred manually when in vivo chlorophyll fluorescence was measured. In vivo chlorophyll fluorescence was monitored at 1–5 h intervals and converted to cell density using equation (1).

Experiment 1 was started 1 h after the lights came on to measure the growth rate during the 14:10 L:D cycle. The growth curve is shown in Fig. 3a. The hourly specific growth rate was 0.54 h^{-1} and was estimated from the regression equation during the 3–9 h incubation during the light period where cell densities increased exponentially. The culture growth stopped before the dark period. Lack of silicate was suspected for the termination of growth because of low 16:1 N:Si and 3:1 Si:P ratios in the medium. However, in a follow-up experiment when silicate was added just after the maximum cell density was obtained, there was no further increase in cell density. Therefore, the growth might have been terminated by lack of CO_2, although the pH did not rise above 8.5.

Experiment 2 was started 10 h after the lights came on to determine if there was growth in the dark. C. salsugineum showed rapid growth during the remaining 4 h of the light period, however, there was little growth in the dark (Fig. 3a; open circles). Experiment 3 was conducted under continuous light. The initial cell density at 0 h was calculated from the dilution of the inoculum because the in vivo chlorophyll fluorescence was too low to detect with the fluorometer. The hourly specific growth rate during the 24 h continuous light incubation was only 0.35 h^{-1} (Fig. 3b) which was 35% lower than that measured in the light period of the 14:10 L:D cycle (Exp. 1), although the daily specific growth rate was still very high at 8.4 d^{-1} because

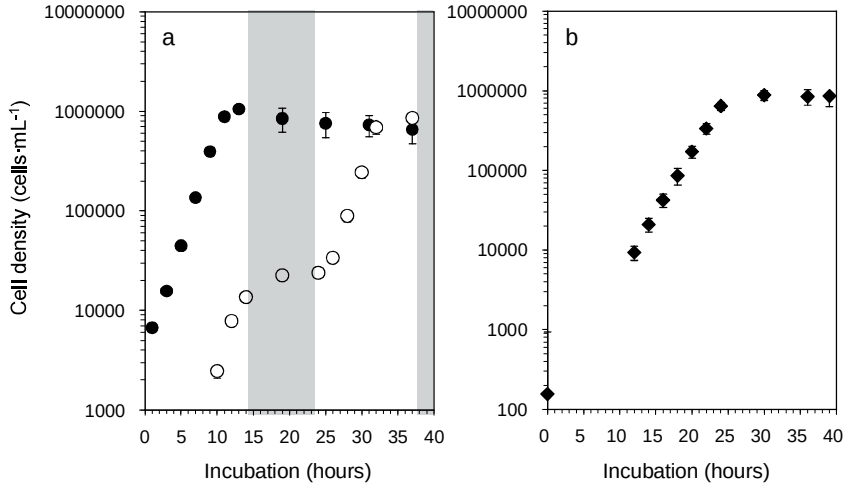

FIG. 3. Growth curve of Chaetoceros salsugineum at 30°C under 700 l mol $\cdot$ m$^{-2}\cdot$ s^{-1}. (a) Closed circles represent growth under a 14:10 L:D cycle and the open circles represent growth starting after 10 h of light and continuing through darkness and into the next light period. The shaded area indicates the dark period. (b) Cell density increase during continuous light. The error bars represent ±1 SD and n = 3.

under the L:D cycle there was little growth in the dark. The decline in growth rate under continuous light has been reported for some other cultures of coastal phytoplankton, especially dinoflagellates (Brand and Guillard 1981).

An increase in the values of other cellular parameters, such as in vitro Chlorophyll a (Chl a) and particulate organic carbon (POC) was also measured. This experiment was started with a very low cell density so that the cell density would not reach a maximum within 24 h. About 1 mL of a culture in log phase growth was inoculated into 1 L of modified ES culture medium in triplicate in the middle of the light period, and incubated at 30°C under 700 l mol · $m^{-2} \cdot s^{-1}$ (14:10 L:D). The initial cell density, Chl a and POC concentration were measured and then again after 24 h. Cell densities were determined microscopically. For Chl a, samples were filtered through a Whatman GF/F and extracted in 90% acetone in the dark at 4°C and Chl a concentrations were determined fluorometrically (Parsons et al. 1984). For POC concentrations, samples were filtered through pre-combusted Whatman GF/F (470°C for 2 h), frozen at) 20°C and measured later on a CHN analyzer (Micro Coder JM-10; J-Science Lab, Co., Ltd, Kyoto, Japan).

Cell density increased from 635 to 629,300 (±74,900) cells · mL^{-1}; Chl a increased from 0.0381 to 36.9 (±4.8) ug · L^{-1} and POC increased from 1.44 to 1,446 (±121) ug · L^{-1} during the 24 h incubation. These results revealed that C. salsugineum increased about 1,000 times in 24 h and the specific growth rate was nearly 7 d^{-1} which was estimated from the equation, $\ln N_2 - \ln N_1/t$, where N_2 and N_1 are the cell densities at 24 and 0 h, respectively and t = time (days).

Eppley (1972) showed that the relationship between maximum growth rates expressed as l_2 (divisions d^{-1}) and temperatures obtained from many published culture experiments for photosynthetic microorganisms could be represented by the following equation; $\log l_2 = 0.0275 \times T$ (°C)) 0.070. When our 30°C incubation temperature was applied to Eppley's equation, the predicted maximum growth rate from the equation was ~5.7 divisions d^{-1} (~4 d^{-1}; converted to ln units). Our results show that C. salsugineum isolated in this study has an exceptionally high growth potential (i.e., almost two times higher than estimated from Eppley's equation for growth at 30°C).

Freshwater chlorophytes and photosynthetic bacteria also have very high growth rates under both high temperature and continuous light. Examples of very high growth rates are: two Chlorophyta, Chlamydomonas mundane (7.6 d^{-1} at 33.6°C) and Chlorella pyrenoidosa (6.4 d^{-1} at 39°C); two cyanobacteria Anacystis nidulans (8.0 d^{-1} at 41°C) and Synechococcus spp. (5.6–6.9 d^{-1} at 37–52°C; Hoogenhout and Amesz 1965). The growth rate of C. salsugineum was very similar to those rates listed above, although the above tested temperatures were beyond the range observed in most parts of the ocean.

Small marine phytoplankton have higher growth rates than larger species (Banse 1982, Geider et al. 1986, Montagnes and Franklin 2001), especially many small Chaetoceros species which have a higher growth potential. For example, C. gracilis grew at 4.2 d^{-1} at 29°C under ~240 l mol · $m^{-2} \cdot s^{-1}$ (24:0 L:D; Thomas 1966). Thompson et al. (1992) showed that C. calcitrans (Paulsen) Takano, C. gracilis Schutt, and C. simplex Ostenfeld had specific growth rates of 3.0–3.5 d^{-1}, 1.5–2.0 d^{-1}, and 2.0–2.5 d^{-1}, respectively at 25°C under 220 l mol · $m^{-2} \cdot s^{-1}$ of continuous light. When C. calcitrans f. pumilus was grown at 25–30°C at a higher light intensity of 500 l mol · $m^{-2} \cdot s^{-1}$ with a 12:12 L:D cycle, the growth rates were 2–3 d^{-1} (Raghavan et al. 2008). Montagnes and Franklin (2001) established an equation for the relationship between specific growth rate of diatoms and temperature and cell volume as follows; l (d^{-1}) = 0.544 + 0.0206 × T (°C)) 0.0864 × log V (l m^3). When a small Chaetoceros species including our C. salsugineum is regarded as a cylinder with a diameter and a height of 5 l m, the cell volume is estimated to be ~100 l m^3 (Hillebrand et al. 1999) and ~1 d^{-1} for the growth rate at 30°C (Montagnes and Franklin 2001). Therefore, the growth rates of these small Chaetoceros species listed above are faster than those rates calculated from the Montagnes and Franklin equations, however, the growth rate of our C. salsugineum isolated from the Shinkawa-Kasugagawa estuary was the highest. The growth potential of C. salsugineum isolated from Amurskii Bay, Sea of Japan, was also measured in general culture conditions (Orlova and Aizdaicher 2000). They showed a typical growth rate of 1.3 d^{-1} at a lower temperature of 20°C and under low light intensities of ~60 l mol · $m^{-2} \cdot s^{-1}$ (12:12 L:D). At present, we do not know whether other C. salsugineum strains isolated from various habitats also have an extremely high growth potential in both high light and temperatures, or whether our isolated clone has unusually high growth rate characteristics.

The C. salsugineum tested in this study was well-adapted to both high temperatures and high irradiances which is rare among coastal and oceanic phytoplankton. In fact, C. salsugineum was observed in various estuarine waters in temperate regions, such as Peter the Great Bay in the eastern coast of Russia (Orlova and Selina 1993, Shevchenko et al. 2004, 2006, Orlova et al. 2009), the Urdaibai estuary in northern Spain (Trigueros and Orive 2001, Trigueros et al. 2002), some brackish lakes (Takano 1983, Ueda et al. 2005), and shallow bays (Takano 1983) along the coast of Japan. Estuarine environments have comparatively higher nutrient concentrations than off-shore waters, and therefore with its very high growth rate, C. salsugineum should be able to out-compete other species under high nutrient and light conditions. Even though C. salsugineum

sometimes occurred at high cell densities, generally it was not normally the dominant species in the observed habitats, including our study site. In nature, net growth rate is determined by the growth rate minus loss rates such as grazing by zooplankters like ciliates. However, our preliminary in situ experiments using natural seawater showed that their grazing rates were insignificant. In our eutrophic estuary, we expect that fast growth may be controlled by some internal or external factors and not by the supply of nutrients.

The C. salsugineum that was isolated in this study has the highest known specific growth rate of any autotroph. Hence, this microalga is an important primary producer in this estuary in the summer. The identification of small Chaetoceros species is difficult because the morphological forms were quite similar, hence the exact distinction between species or strains from various habitats should be determined in the future using molecular techniques. Future research will explore how C. salsugineum utilizes high light energy for photosynthesis so efficiently and examine the physiological mechanisms it uses to achieve high speed cell division combined with genetic analysis.

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