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Isolation of high-level-CO_2-preferring *Picochlorum* sp. strains and their biotechnological potential

Kazuki Watanabe [a], Katsuhiko Fujii [a,b,*]

[a] Graduate School of Agriculture, Yamaguchi University, Yoshida, Yamaguchi 75385151, Japan
[b] Graduate School of Science and Technology for Innovation, Yamaguchi University, Yoshida, Yamaguchi 75385151, Japan

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ABSTRACT

Production of CO_2-tolerant microalgae have received much attention as well as physicochemical fixation of CO_2 in industrial flue gas. Although many microalgae that are tolerant to high levels of CO_2 have been found and evaluated, the CO_2 concentration for their good growth is generally lower than their maximum tolerable CO_2 level. In the present study, we attempted to isolate microalgae capable of growing in high levels of CO_2 (high-level-CO_2-preferring microalgae, HCP-microalgae). We used a CO_2-permeable polystyrene bottle for the enrichment of HCP-microalgae in environmental samples. Two *Picochlorum* strains, Azisu1 and Azisu2, were isolated following enrichment of seawater. The strains showed fast growth in 20%–40% CO_2 and contained high amount of some vitamins (B1, B6, and C), L-proline, and γ-aminobutyric acid. The C18 unsaturated and C16 saturated fatty acids were found dominant. In addition, astaxanthin was also found, but its cellular content was not high. Based on the previous reports on some *Picochlorum* sp. strains, it can be concluded that Azisu1 and Azisu2 may be promising candidates for commercial applications in nutraceutical, livestock, and aquaculture industries.

1. Introduction

Increase in atmospheric CO_2 level is thought to be one of the main causes of global warming [1]. The United Nations Framework Convention on Climate Change had indicated the necessity among developed nations to reduce CO_2 emission, and the member nations in the convention had discussed the establishment of carbon tax or emission trading [2]. A major source of emitted CO_2 is industries, and the energy sectors account for approximately 75% of the total CO_2 emission [3].

Some technologies for CO_2 reduction are currently under development. Carbon capture and storage (CCS) is the process of capturing CO_2 in industrial exhaust gas, transporting it to a storage site, and depositing it underground [4]. Although the key technologies for CCS are available, cost reduction, improved CO_2 monitoring, as well as detailed evaluation of the environmental impact of deposited CO_2 are indispensable for its practical application [4,5]. Carbon capture and utilization (CCU) is regarded as an alternative strategy, which comprises capturing CO_2 and converting it to commercially valuable products [6]. However, the chemical conversion of CO_2 to valuable products in this process does not have commercial viability, due to drawbacks including high production cost and requirement of highly selective catalysts for manufacturing of the products.

In contrast, biological conversion of CO_2 to value-added products by microalgae is an attractive strategy because of its advantages such as low production cost, ability to fix CO_2 directly from the exhaust gas, and not requiring catalysts [6]. In fact, several microalgal mass cultures have been commercialized and utilized mainly as raw materials for aquaculture feed and nutraceuticals [7]. However, they are usually cultivated under atmospheric air containing only 0.04% of CO_2 because supplementation of excess CO_2 results in acidification of the culture medium and inhibition of algal growth [8]. Flue gas from coal-fired power plants, the largest CO_2 source, contains 12%–15% of CO_2 [9], and therefore microalgae that can grow in high levels of CO_2 can be employed for the bioconversion of CO_2 in the flue gas to valuable products.

There are many reports on cyanobacteria or eukaryotic microalgae that are tolerant to high concentration of CO_2 (>10%) (Table 1), with some strains known to survive even in 100% CO_2, suggesting a vestigial characteristic of ancestral photosynthetic microbes that lived in the Early Earth [10]. However, the CO_2 concentration for their good growth is generally lower than the maximum CO_2 concentration that they can tolerate (Table 1), and the former should be regarded as more important to evaluate the feasibility of culturing algal strains in high concentrations of CO_2. Thus, it is attractive to screen or isolate microalgal strains capable of demonstrating fast growth in high levels of CO_2 (high-level-CO_2-preferring microalgae, HCP-microalgae) from the standpoint of paleontology as well as biotechnological potential. To the best of our knowledge, the highest CO_2 concentration in which HCP-microalgal strains showed good growth is around 20%

* Corresponding author.
E-mail address: kfujii@yamaguchi-u.ac.jp (K. Fujii).

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Table 1
Known CO_2-tolerant cyanobacteria and eukaryotic microalgal strains.

Strain	Taxonomy	Origin	Max CO_2 tolerated (v/v %)[a]	CO_2 level showing good growth (v/v %)[a]	Reference
Chroococcidiopsis sp.	Cyanobacterium	Hot spring	60	20	[35]
Synechococcus sp.		Freshwater	55	Not tested	[10]
Anabaena sp.			55	Not tested	[10]
Plectonema boryanum			100	Not tested	[10]
Synechococcus elongatus		Seawater	60	5	[36]
Cyanobacterium spp.			>20	10	[37]
Cyanidium caldarium	Eukaryotic alga	Hot spring	100	Not tested	[38]
Chlorella sp.		Freshwater	>40	20	[39]
Chlorella kessleri			>18	18	[40]
Chlorella sp.			>70	10	[41]
Chlorella sp.			80	15	[42]
Scenedesmus obliquus			>18	18	[40]
Scenedesmus sp.			80	10	[43]
Scenedesmus spp.			>20	10	[37]
Chlorococcum littorale		Seawater	60	5	[44]
Nannochloris spp.			>20	10	[37]
Chlamydomonas sp.			>15	5	[45]
Desmodesmus sp.			100	<5	[46]

[a] Values were obtained from published literatures.

(*Chroococcidiopsis* and *Chlorella* spp. in Table 1), although that for some cyanobacteria and *Cyanidium* sp. strains are not well documented. Thus, in the present study, we attempted to isolate and characterize HCP-microalgae which show fast growth in >20% CO_2 concentration. In particular, we focused on the isolation of marine microalgae because the ocean is the largest habitat of microalgae but the ecology of CO_2-tolerant algae is not well understood as that of the freshwater ones. In addition, the biotechnological potential of the HCP-strains obtained in this study was also evaluated.

2. Materials and method

2.1. Chemicals

Chemicals including salts, vitamins and organic solvents were purchased from Wako Pure Chemicals (Osaka, Japan). Marine Art SF-1, the artificial seawater, was purchased from Tomita Pharmaceuticals (Tokushima, Japan). Molecular biology reagents were obtained from Toyobo (Osaka, Japan).

Table 2
Compositions of the culture medium (NAS) used in this study.

Constituents	Medium composition (per liter)
NaCl	22.1 g
$MgCl_2$ $6H_2O$	9.9 g
$CaCl_2$ $2H_2O$	1.5 g
Na_2SO_4	3.9 g
KCl	0.61 g
$NaHCO_3$	0.19 g
KBr	96 mg
$Na_2B_4O_7$ $10H_2O$	78 mg
$SrCl_2$	13 mg
NaF	3 mg
LiCl	1 mg
KI	81 mg
$MnCl_2$ $4H_2O$	0.6 mg
$CoCl_2$ $6H_2O$	2 mg
$AlCl_3$ $6H_2O$	8 mg
$FeCl_3$ $6H_2O$	5 mg
Na_2WO_4 $2H_2O$	2 mg
$(NH_4)_6Mo_7O_{24}$ $4H_2O$	18 mg
$NaNO_3$	7.5 g
NH_4Cl	2.67 g
NaH_2PO_4 $2H_2O$	0.6 g
Thiamine HCl (VB1)	0.2 mg
Biotin (VB7)	1 mg

2.2. Culture medium

Nutrition-supplemented Artificial Seawater (NAS) medium, the culture medium used in this study, was prepared by supplementing nitrogen, phosphate, vitamins, and soil extract to 38.2 g l^{-1} Marine Art SF-1 solution. The chemical composition of the culture medium is shown in Table 2. The medium was sterilized by filtration using a 0.22-μm Stericup filter unit (Millipore, Massachusetts, USA) because autoclave treatment resulted in precipitation.

2.3. Enrichment of HCP-microalgae

To isolate marine microalgal strains, seawater samples were collected from four coastal areas in Yamaguchi, Ube, and Hagi cities, Yamaguchi Prefecture, Japan (Table 3). Each sample (1000 ml) was filtered through a 0.22-μm Millipore Omnipore membrane, and the residue left on the filter was suspended in 50 ml of NAS medium and recovered in a 150-ml polystyrene bottle (Corning, New York, USA), which was bubbled with pure CO_2 for 1 min and tightly sealed. The bottle was shaken on a reciprocal shaker (120 rpm) at 25 °C for 4 weeks with illumination provided by fluorescent lights (30 μmol photon m^{-2} s^{-1}) to enrich the HCP-microalgae.

2.4. Isolation of algal strains

An aliquot (2 ml) of the grown subculture was serially diluted (10^1–10^5 fold dilutions) in the NAS medium to obtain a cell suspension containing 1 microalgal cell per ml and then directly dispensed into a 96-well microplate (150 μl per well). The microplates were then put in a polycarbonate rectangular container, in which pure CO_2 was flushed for 1 min before sealing, and incubated at 25 °C for 4 weeks with illumination provided by fluorescent lights (30 μmol photon m^{-2} s^{-1}). The culture grown from 10^5-fold diluted aliquot in a microwell was recovered, propagated again in 50 ml of NAS medium in a polystyrene bottle, and its species homogeneity was confirmed based both on microscopic and phylogenetic analyses.

2.5. Growth test for microalgae under various CO_2 concentrations

The algal preculture was mixed with fresh NAS medium to obtain cell density of 1.0×10^6 cells ml^{-1}. A total of 10 ml of the cell suspension was added to a 50-ml glass vial (Maruemu, Osaka, Japan) that was flushed with different concentrations of CO_2 (0.04%, 2%, 20%, 40%, 70%, and 100% v/v) at the flow rate of 2 ml min^{-1} for 1 min and sealed