

Characteristics of the released single cells
from colonial oil-producing green alga
Boryococcus braunii and expression cassettes
for transformation

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その他のタイトル	油生産性群体緑藻ボトリオコッカス ブラウニーの 遊離単細胞の特徴と形質転換のための発現カセット 構築
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論文内容の要旨

Recently biofuel production from microalgae have attracted attention. Many oil-producing green microalgae have been isolated, and cytochemical characteristics have been studied for some species. Among oleaginous microalgae, colonial green alga *Botryococcus braunii* (*B. braunii*) is one of the most promising species. *B. braunii* is classified into three biochemical races: A, B, and L, according to the types of primal hydrocarbon oils they produce. In stationary phase, the lipid ratio reaches to 86% of its dry weight. In B-race, primary oil is a hydrocarbon named botryococcene, and 90-95% of the hydrocarbons are stored in the colonial extracellular matrix.

B. braunii is a brilliant candidate for fossil fuels substitute, however, the growth rate of most strains are extremely slow, and oil production level varies significantly among strains or in culture conditions. Thus, to accomplish the remarkable production of hydrocarbons at a reasonable price, application of molecular biological techniques for *B. braunii* is essential. However, accumulated hydrocarbon oils in the extracellular matrix hamper it, while single cells that are free of the oils would be extremely useful for genetic manipulation of this alga.

Till date, only solid heavy metals such as gold or tungsten have been used as DNA carriers in biolistic bombardment of algae. In this study, I showed that even a metal oxide of lower density can act as a DNA carrier. I investigated the potency of size-controlled mesoporous titanium dioxide (TiO_2) particles. Highest transformation was shown at 1,100 psi (approximately 7.6 mPa) and 2,000 psi (approximately 14 mPa) for the green alga *Chlamydomonas reinhardtii*. Surprisingly, a mesoporous metal oxide with a density of approximately only one-tenth that of gold or tungsten could be effective as a DNA carrier in biolistic bombardment of a rigid cell wall-containing alga. In addition, I found two peaks of gas pressures in the transformation ratio irrespective of whether the particles were made of gold, tungsten, or TiO_2 .

The detailed methodology and the characteristics of the prepared single cells were investigated in this study. Four different varieties of *B. braunii* that derive from different races, *Showa*, *Sanshiro-5*, *UTEX572*, and *Yamanaka*, were studied. Chemical reagents that would be useful in preparing a large number of vital single cells from colonial *B. braunii* were tested. Among the 18 reagents assayed, as many as 10 reagents showed potency for releasing *Showa* single cells (B-race); 8 reagents showed potency for releasing *Sanshiro-5* single cells (B-race); and only 3 reagents showed potency for

releasing *Yamanaka* single cells (A-race). However, the potent reagents did not share any apparent chemical similarities. Fluorescent staining with Nile red revealed that the released single cells were free of extracellular hydrocarbon oils. Brightener 28 staining, which is a cellulose specific fluorescent dye, showed the presence of cell wall around released single cells. While, to maintain the prepared single cells in vital condition, they must be maintained at a high concentration ($>2 \times 10^7$ cells/mL); at low concentrations, they rapidly lost chlorophyll and get disrupted. In contrast to the above results obtained using *Showa* (B-race), single cells prepared from *UTEX 572*, which is a A-race variety, survived even at low cell concentrations.

Since transformation has been proved to be feasible in chlorophyte algae such as *Chlamydomonas reinhardtii*, those isolated single cells must be the best material for transformation. In addition to that, single cells must be useful for other molecular engineering methods, such as cell fusion or mutagenesis. DNA constructs that domestic promoters drive phleomycin resistance (*ble*) gene or green fluorescent protein (*gfp*) gene were generated to transform prepared single cells of *Showa* and *UTEX572*. Various methods of transformation were tried to obtain stable transformants.