Characterization of isolates derived from heavy-ion-beam irradiated cells in the unicellular green alga *Parachlorella kessleri*

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Parachlorella kessleri is a unicellular microalga relatived to the *Chlorella* species that belong to the class of Trebouxiophyceae in the Chlorophyta and accumulates starch followed by lipids after suffering sulfur-starvation. [1)] A *P. kessleri* strain (NIES-2152) exhibits the highest biomass productivity under continuous high-light conditions among the strains that are relative algal species. [2)] The properties of *P. kessleri* such as the ability to produce biomaterials including starch and lipids, and high biomass productivity qualify this strain as the most desired candidate in industrial production.

To improve the productivity of biomass or biomaterials in algal cells, we have been developing a breeding system for microalgae using *P. kessleri* as a model case. Our previous report demonstrated that the isolates from heavy-ion-beam irradiated *P. kessleri* cells exhibit a broad spectrum of phenotypes, which are different from the wild type strain. [3)] Another case study demonstrated that the heavy-ion-beam irradiation could disrupt gene(s) responsible for a metabolic pathway to assimilate extracellular nitrates by transporting and oxidizing them to ammonium. [4)] These findings suggest that the breeding system based on the heavy-ion-beam will modify a specific metabolic pathway to produce biomaterials.

Wild type *P. kesseleri* cells were grown in the wells of a 384-well plate using a tris-acetate-phosphate (TAP) medium containing inorganic nutrients sufficient for healthy growth under continuous-light conditions. Optical density at 595 nm (OD_{595}) and the emission of a lipophilic fluorescence stain, Nile Red, in each well were measured as an index of cell density and accumulation of neutral lipids in cells, respectively (Fig. 1). The median of OD_{595} for wild type *P. kessleri* cells reached to approximately 1.11 at 12th day of culture after inoculation into a fresh TAP medium. Although the individual values were scattered, they exhibited a constant range of scatter in replications. The wild type cells were grown in nutrient-limited media (dSTAP for sulfur starvation; dNTAP for nitrogen starvation; dPTAP for phosphorus starvation). Among the three conditions, nitrogen starvation suppressed the increase of OD_{595} after inoculation most effectively. In addition, the values in individual wells exhibited also a constant range of scatter. The Nile Red fluorescence values for the three nutrient-limited culture media were almost the same, and they were higher than that for the TAP-medium, suggesting that the neutral lipids accumulate in those cells in high and

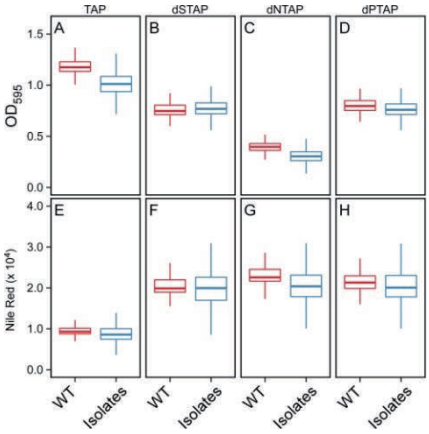

Fig. 1. Boxplots of OD_{595} values and Nile Red emissions in replications for the wild type strain and isolates from heavy−ion−beam irradiated *P. kessleri* cells.

isolates using this method should consider the scatter between wells.

The *P. kessleri* cells were irradiated by heavy-ion beams of different doses and nuclear species (Fe and Ar ions at 25 Gy and 50 Gy). These cells were grown on plates containing a solid TAP medium, and the obtained colonies were defined as isolates. The individual isolates were inoculated in the wells of 384-well plates containing a fresh TAP medium. Four thousand six hundred and eight isolates were grown for 12 days. OD_{595} values and Nile Red emissions for these wells were measured at the 12th day of culture after inoculation (Fig. 1). The distribution of OD_{595} values differed from the expected pattern with the constant range of the scatter spreading around the median calculated for the wild type replications. Although the exact scatter of individual isolates was unclear because the value was measured only once, this result indicates that the population contains heterogeneous isolates with different genetic backgrounds. Under sulfur and phosphorus starvations, the scatters were consistent with that for the wild type replications; however, under nitrogen starvation the median was lower than that for the in the case of wild type. These inconsistencies with the expected result were observed in the constant range of scatter for Nile Red emission except in the case of sulfur starvation. These data suggest that a certain group of isolates from heavy-ion-beam irradiated algal cells alters the genetic background responsible for cell proliferation and metabolism.

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