Increase of lipid production upon outdoor cultivation of heavy-ion beam irradiation mutant *Parachlorella kessleri* PK4 and identification of its genetic variations†

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In recent years, microalgae have gained significant attention as a promising feedstock for the biofuel industry. Some microalgae such as *Parachlorella kessleri*, a species separated from the genus *Chlorella*, can accumulate high levels of starch and lipids that can be used for the production of bioethanol and biodiesel, respectively.[1,2] Irradiation of *P. kessleri* with heavy-ion beams with varying doses and ion species resulted in the generation of phenotypes with potential economic relevance in terms of lipid production.[3] One such mutant, PK4, exhibited high lipid accumulation under nitrogen starvation conditions.[3]

In this study, the lipid accumulation of PK4 was analyzed using two experimental systems: a laboratory-based small-scale culture and thin-layer photobioreactor (T-PBR) mass cultivation in a culture volume of 150 l. Nutrient dilution was adopted to maximize the lipid productivity by exploiting the PK4 response to nutrient limitation. We also determined its genetic variation by whole genome re-sequencing.

TAP and UP media were compared to maximize the lipid productivity of PK4 at a laboratory scale (80 ml). The growth of wild type (WT) and PK4 stopped in the complete TAP media on day 6 post-inoculation, but both WT and PK4 continued to proliferate in the UP media even on day 8 post-inoculation. In the complete UP media, only a small amount of lipid accumulated in either WT or PK4 cultures. The culture on day 4 post-inoculation was collected and diluted with sterilized distilled water and individual cultivations were continued. PK4 accumulated more lipids and at an earlier stage than WT in UP medium after nutrient dilution.

The nutrient dilution experiment was performed in a 150 l thin layer- photobioreactor (T-PBR) (Fig. 1). The culture was diluted with water four times to induce nutrient limitation on day 7 post-inoculation. Lipids began to increase from day 9 post-inoculation (2 days after dilution) and continued to increase until the last day of cultivation (38% DW, 2.0 $g \cdot L^{-1}$). The maximum lipid content was 66% per dry weight (Day13, 2.9 $g \cdot L^{-1}$) and the maximum lipid productivity after dilution was 0.59 $g \cdot L^{-1} \cdot day^{-1}$.

The locations of genetic variation in PK4 were investigated by next-generation sequencing (Illumina MiSeq and HiSeq). In total, we identified three loci, which probably caused the observed phenotype in the automated mutation analysis pipeline (AMAP) with some modifications.[4] They correspond to putative homologs of elicitor-responsive protein (9934_t), ATP/ADP transporter (9067_t) and endo-1,4-β-mannanase (8741_t). There are three copies of the 9067_t gene in the genome. One of the three homologs is ATP and ADP transporter localized in the chloroplast envelope. However the relationship of this transporter with membrane lipids has been discussed.[5] The 8741_t gene has 13 genetic copies. Mannan is one of the polysaccharides present in the cell wall of algae.[6] 1,4-β-mannanase hydrolyzes β-1,4-mannosidic linkages of D-mannan, as well as galacto- and glucomannans at random site.[7] A study of metabolites or genetics including complementation of the affected genes would be required to determine the gene(s) whose mutation is responsible for the PK4 phenotype.

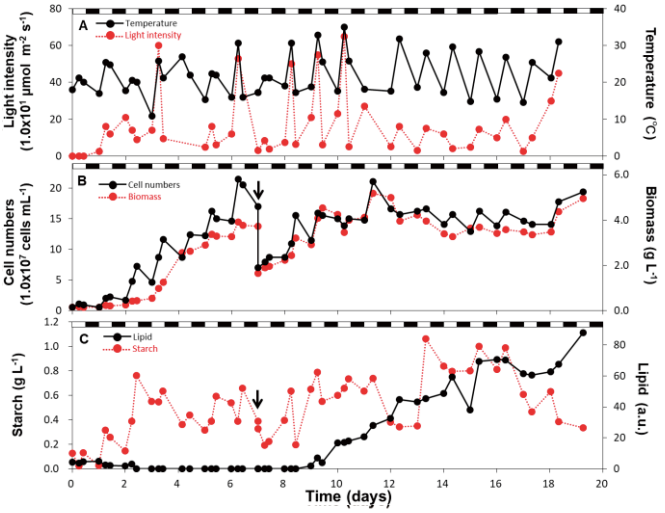

Fig. 1. Time-series data in T-PBR. (A) water temperature and light intensity, (B) cell numbers and biomass, and (C) content of starch and lipids. The culture was diluted with water four times on day 7 post-inoculation.

References
1) B. Fernandes *et al.*, Bioresour Technol. **144**, 268–274 (2013).
2) X. Li, *et al.*, Biotechnol Bioeng. **110**, 97–107 (2013).
3) S. Ota *et al.*, Bioresour Technol. **149**, 432–438 (2013).
4) K. Ishii *et al.*, Syst. **91**, 229–233 (2017).
5) A. G. Navarro *et al.*, J. Cell. Physiol. **229**, 2126–2136 (2014).
6) H. Takeda *et al.*, Phytochemistry **34**, 1053–1055 (1993)
7) Do Bien-Cuong *et al.*, Factories **8**, 59 (2009).

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