Mutation rates of *Parachlorella kessleri* by heavy-ion-beam irradiation and classification of their genomic deletion types

T. Yamazaki,[*1,*2] S. Ota,[*1,*2] K. Oshima,[*1] M. Hattori,[*1] Y. Kazama,[*3] T. Abe,[*3,*4] S. Kawano,[*1,*2]

Consumption of fossil fuels such as petroleum oil not only depletes a finite resource but also increases carbon dioxide levels, which causes global warming. Because of carbon fixation, plants can contribute to the reduction of carbon dioxide levels. Moreover, biofuels from plant have been considered as substitutes for fossil fuels. Microalgae, which perform photosynthesis like terrestrial plants, have been attracting attention as a feedstock of biodiesel production in recent years. To increase the production of biomass and improve the amount of biofuel from microalgae, the common process used is the isolation of strains with high biomass productivity and/or high oil content from the natural environment and optimization of culture conditions for the isolated strains. However, this strategy does not necessarily obtain the best strains. Therefore, we artificially modified the genome of microalgae by using the heavy-ion-beam irradiation. The heavy-ion beam is considered a mutagen that causes the partial deletion of genomic DNA very effectively, and it can also be used to breed a variety of organisms, including flowering plants. Because little is known about breeding of unicellular microalgae by using heave-ion-beam irradiation, evaluation of its effectiveness is necessary.

In this study, we used a green microalga, *Parachlorella kessleri*, the draft genome of which has been determined by our group. A search for the draft genome sequence revealed that *NR*, *NRT* and *NIR* are single-copy genes, and *NAR* is duplicated into two genes (*NAR1-1*, *NAR1-2*). The transcript of *NAR1-1* was not detected in transcriptome data, suggesting that nitrate is metabolized to ammonia through a single pathway (NRT-NR-NAR-NiR pathway) in *P. kessleri* (Fig. 1A). Therefore, a *P. kesseleri* mutant that can grow in an ammonia-containing medium but not in a nitrate-containing medium is considered to have a defective in the nitrate assimilation pathway.

P. kessleri cells were irradiated by heavy-ion beams of different doses and nuclear species, and then screening was performed using following steps: (1) a single clone derived from irradiated *P. kessleri* cells was inoculated in a TAP medium containing ammonia[1]; (2) After the *P. kessleri* cells were grown, they were inoculated in both TAP medium and a modified TAP medium containing only nitrate as a nitrogen source; (3) a putative mutant grow in the TAP medium, but not in the modified TAP medium. Doses and nuclear species used for the screening are carbon ions the

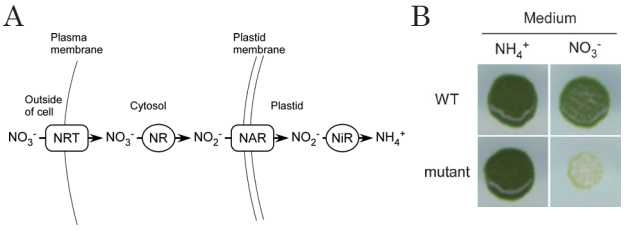

Figure 1. (A) Nitrate assimilation pathway. NRT, nitrate transporter; NR, nitrate reductase; NAR, nitrite transporter; NiR, nitrite reductase. (B) Phenotype of the mutant requiring annmonium. Wild type and mutant *P. kessleri* cells of wild type and mutant were grown on a medium containing ammonium or nitrate. After one week, their colonies were photographed.

(50 Gy, 25 Gy) and argon ions (25 Gy, 50 Gy). Fig. 1B shows a representative phenotype of the mutant isolated from the heavy-ion-beam irradiated mutant that requires ammonia as a nitrogen source.

A large-scale of screening was performed, and the mutants were isolated from about 4000 clones for 25 Gy and 50 Gy of argon-ion beams, and 25 Gy and 50 Gy carbon-ion beams, respectively. As a result, the mutants requiring ammonia from the individual doses and nuclear species were obtained each 0, 4, 3 and 4, respectively. The incidence in each experiment was 0.00, 0.10, 0.07, and 0.10% (Table 1). In the future, we plan to calculate the mutation rate of the irradiation by iron-ion beams.

Table 1. Mutation frequency by heavy-ion-beam irradiation

Nuclear species	Dose (Gy)	Isolated clone number	Positive clone number	Frequency (%)	Survival rate (%)*
C	25	4005	0	0.00	n.d.
	50	4007	4	0.10	59
Ar	25	4007	3	0.07	50
	50	4012	4	0.10	28

*Ota et al. (2013)

Next, the PCR fragment analysis and restriction fragment length polymorphism-PCR analysis were performed for the *NR* gene. However, there was no difference between the wild-type and all mutants, suggesting that the deletions of genomic DNA by heavy-ion-beam irradiation are very small in microalgae. Currently, we are performing re-sequence analysis using the next generation sequencer IonProton for the 11 mutants, expecting a detailed comparison of the deletion pattern of a set of genes involved in nitrate metabolism, including the *NR* gene.

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References

1) Ota et al. : Bioresour Technol. 149:432-438. (2013)

[*1] Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo
[*2] JST, CREST
[*3] RIKEN Innovation Center
[*4] RIKEN Nishina Center