Chromosomal rearrangement induced by high-LET heavy-ion-beam irradiation in *Parachlorella kessleri*

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Heavy-ion beams are used as an effective mutagen that induces localized mutations owing to their high linear energy transfer (LET).[1)] Breeding with heavy-ion beams has been attempted often on land plants and recently on microalgae. *Parachlorella kessleri* (Chlorellales, Trebouxiophyceae, Chlorophyta) is a type of unicellular green algae that has received much attention as a biological resource for biomass production. A *P. kessleri* mutant with high oil production has been produced by heavy-ion-beam irradiation.[2)] Recently, in a land plant *Arabidopsis thaliana*, it has been reported that an Ar ion beam (LET: 290 keV/μm) significantly induced chromosomal rearrangements more frequently than a C ion beam (LET: 30.0 keV/μm).[3)] In this study, we produced *P. kessleri* mutants in which chromosomes were fragmented by Ar-ion-beam and Fe-ion-beam (LET: 640 keV/μm) irradiations. We performed mutation analysis on those mutants to characterize the nature of mutations induced by the high-LET heavy-ion beams.

P. kessleri was irradiated with the Ar-and Fe-ion beams with a dose of 75 Gy and cultured for 16–20 h in a TAP medium at 23°C under the continuous light condition. For microscopic observation, both irradiated and unirradiated cells were fixed by glutaraldehyde and stained by SYBR Green I. A typical karyotype of the wild type indicates that *P. kessleri* has seven chromosomes (Fig. 1A). On the other hand, chromosome fragmentation was observed in the irradiated cells (Fig. 1B–C). Irradiated cell lines were established by single colony isolation. Though some of these fragmented chromosomes were not inherited by descendant cells, the two established lines (Fe75-1-3H and Ar75-1-3H) were confirmed to stably possess fragmented chromosomes by pulsed field gel electrophoresis (data not shown).

The two established lines (Fe75-1-3H and Ar75-1-3H) and the wild-type line were resequenced by the MiSeq sequencing system (Illumina Inc., `https://www.illumina.com`). Mutation analysis was conducted by the mutation analysis pipeline AMAP as described previously[4)] with some modifications: we modified AMAP to utilize the draft genome sequence (consisting of 400 scaffolds and 60 Mb in total) and the gene structure information (in the GTF format) of *P. kessleri*. As rearrangements defined previously[3)] as including indels more than 100 bp and chromosomal rearrangements, one inversion was detected in Ar75-1-2C. In Fe75-1-3H, two inversions and three translocations were detected. All of the three translocations were localized in a region of approximately 2 kb of the genome, which

Fig. 1. Karyotypes of *P. kessleri*. (A) Typical karyotype of the wild type. (B–C) Typical karyotypes of cells after irradiation with Ar- (B) and Fe-ion beams (C), indicating that chromosomes were fragmented into 17 and 39 parts, respectively.

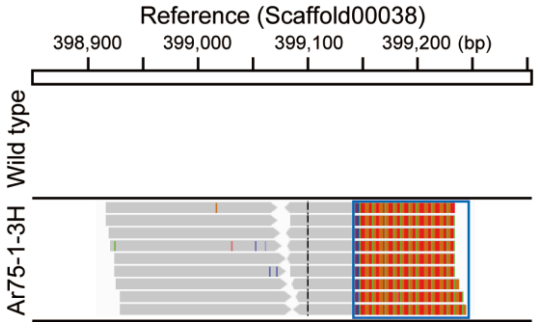

Fig. 2. Mapped read sequences including only telomeric repeats. The gray bars indicate read sequences. The colored lines on read sequences indicate single nucleotide polymorphisms. The blue box indicates telomeric repeats.

may reflect the dense ionization produced by the high-LET heavy-ion beam. To detect chromosome rearrangements involving chromosome ends, reads that only include continuous (more than three units) telomeric repeat (TTTAGGG) were extracted from the resequenced data and mapped to the draft genome sequence by Burrows-Wheeler Aligner. One newly formed junction with telomeric repeats in Ar75-1-2C was found (Fig. 2). This junction possibly explains the stability of some of the fragmented chromosomes. Alternatively, it was possibly the junction with internal telomere sequences. Fluorescence *in situ* hybridization that utilizes the genomic sequence near the junction as a probe will provide information based on which a hypothesis would be plausible.

In this study, it was revealed that the Ar and Fe ion beams induced chromosome fragmentations in *P. kessleri*. Mutation analysis suggested that the fragmentations were caused by localized DNA double-strand breaks and that chromosome ends of stable fragmented chromosomes possessed telomeric repeats. The complete genome sequence will provide clearer information on the nature of mutations induced by the high-LET heavy-ion beam.

References

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