P2-2 Project “Ion Beam Mutagenesis”

Leader : OONO Yutaka

Ion beams are useful mutagens for plant and microbes because they are thought to cause mutations via a mechanism distinct from those of chemical mutagens or gamma rays. Our project aims to understand feature of ion-beam-induced mutations and develop applications of ion beam mutagenesis in basic and applied biotechnology. Recently, we use a next generation sequencing technique to investigate the detailed characteristics of the ion-beam- and gamma-ray-induced mutations at both genome wide and specific gene levels {2-08, 2-11 in Part II}. In addition, we try to isolate valuable mutants in ornamental plant, parasitic plants, plant growth-promoting rhizobacteria, oil-producing algae, sake yeasts, and other bacteria under collaborations with academic or industrial research organizations {2-09, 2-10, 2-12, 2-13, 2-15}. Revealing molecular basis of radiation response and resistance is another major business in our project {2-14, 2-16~18}.

Non-homologous end joining (NHEJ) is an important repair pathway that protects the genetic information in plant genome [1]

NHEJ is one of two major pathways to repair DNA double-strand breaks. Because it directly re-joins the two broken DNA ends, it is thought more mutagenic than another major DSB repair pathway, homologous recombination that uses sequence information of a homologous DNA strand for repairing. To investigate the role of NHEJ in radiation-induced mutagenesis, Yan Du, a visiting researcher from China under Nuclear Researchers Exchange Program, with collaborators including two our project members, Yoshihiro Hase and Katsuya Satoh, conducted whole genome sequencing analysis of gamma-ray induced mutations in two Arabidopsis mutants (*Ku70*$^{-/-}$ and *Lig4*$^{-/-}$) that luck NHEJ DNA repair components. A dose repose curve of survival suggested that the NHEJ-deficient mutants are hypersensitive to gamma rays and that 100 Gy for the NHEJ-deficient mutants is equivalent to 1000 Gy for wild type plants. Thus, the seeds of the NHEJ-deficient mutants were treated with 100 Gy gamma rays followed by the mutation profile in the M2 plants of the NHEJ-deficient mutants was investigated and compared with previously obtained data of the wild type plants irradiated with 1000 Gy.

As shown in Fig. 1, the mutation frequency (number of mutation / base / Gy) in the NHEJ-deficient mutants was higher than that in the wild type plants. In addition, the deletions (≥ 2 bp) and complex-type mutations were frequently detected in the both mutants. These results suggest that NHEJ deficiency results in arising severe mutations as well as increasing sensitivity to gamma rays. In other words, NHEJ is required for precise repair of broken DNA and has significant role to minimize the deleterious effects of ionizing radiations in plants.

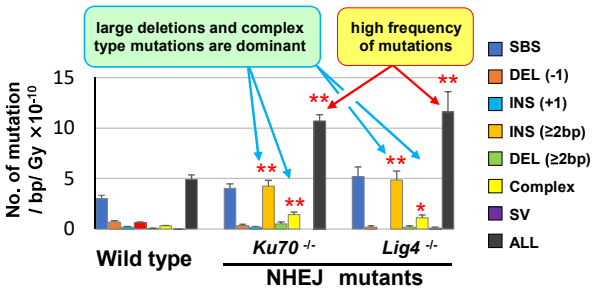

Fig. 1. Mutation frequency (number of mutation / base / Gy) in the wild type and two NHEJ-deficient mutants (*Ku70*$^{-/-}$ and *Lig4*$^{-/-}$).
Asterisks indicate significance to wild type (**p < 0.01 and *p < 0.05). SBS, single base substitutions; DEL, deletions; INS, insertions; SV. structural variants. Figures in parentheses represent size (bp) of DEL or INS.

Uncovering genome of the cesium-accumulating bacterium [2]

The bacterium *Rhodococcus erythropolis* CS98, recently reclassified as *Rhodococcus qingshengi*, is expected to be useful for removing radioactive cesium from contaminated soil because this bacterium has high ability to accumulate the cesium in the cell. Exploring cesium accumulation mechanism in this bacterium is also helpful to further improve the ability of cesium accumulation and develop practical strains for radiocesium bioremediation. In this context, Satoh *et al*. completed genome sequencing of *R. qingshengii* CS98 by both long- (GridION) and short-read (MiSeq) genome sequencing methods. Sequences obtained by GridION (1,389,142,401 bp) and MiSeq (259,666,780 bp) were assembled and polished to construct the genome of *R. qingshengii* CS98, which was comprised of one circular chromosome (6,240,414 bp) and one linear plasmid (485,693 bp). Its total length was 6,726,107 bp with 62.4% G+C content on average. Annotation pipeline predicted 6,255 protein-coding sequences (CDSs), 59 tRNAs, and 5 rRNA operons (Table 1). The linear plasmid possessed plasmid partitioning gene *parA*. The chromosome sequence of the CS98 was highly similar to that of *R. qingshengii* strains djl-6-2 (99.07% identity) and RL1 (99.09% identity). Furthermore, the linear plasmid showed a high level of similarity (99.15% identity) to the linear plasmid of the *Rhodococcus* sp. strain BH4. Further comparative genome analyses will help to elucidate the molecular basis of cesium accumulation in the bacterial cells.

Table 1
General feature of complete genome sequence of *R. qingshengii* CS98.

	Chromosome	Plasmid
Size (bp)	6,240,414	485,693
G+C content (%)	62.54	60.41
No. of CDSs	5,811	444
No. of tRNA	58	1
No. of rRNA (5S / 16S / 23S)	(5 / 5 / 5)	(0 / 0 / 0)

References

[1] Y. Du *et al*., J. Radiat. Res. **61**, 639 (2020).
[2] K. Satoh *et al*., Microbiol. Resour. Announ. **9**, e01188-20 (2020).