P2-1 Project "Ion Beam Mutagenesis"

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The ultimate goal of our project is to develop applications of quantum beam technology in applied biological fields such as sustainable agriculture and environmental conservation. Ion beams are recognized as useful mutagens for plant and microbe breeding because they are thought to cause mutations by distinct mechanism from chemical mutagens or gamma rays. To develop more efficient ion-beam mutagenesis techniques, we have tried to understand the characteristics of the ion-beam-induced mutations by using specific gene markers or genome-wide sequencing {2-10, 12, 21, and 22 in Part II}. In addition, under collaborations with academic or industrial research organizations, we are aiming to isolate valuable mutants in various organisms such as parasitic plants, plant growth-promoting rhizobacteria, oil-producing algae, sake yeasts, and other bacteria by ion-beam irradiation {2-11, 14, 16~19, and 25}. Revealing molecular biology basis of radioresistant organisms is another major business of our project {2-15, 20, and 26}.

Genome sequence of a radioresistant bacterium in the sky above Japan [1]

Bacteria classified in the genus *Deinococcus* are well known as radioresistant bacteria (Fig. 1). They live almost everwhere in the world. Approximately 70 *Deinococcus* species have been isolated from various enviroments such as soil, hot springs, foods, faeces, air-borne dust, alpine environments, activated sludge, freshwater, and rhizosphere. How they protect and repair DNA from highly harmful ionizing radiation is a very attractive issue to be clarified. Recently, comparative genome approach is getting powerful to elucidate the special features of *Deinococcus* bacteria in genomic level. Satoh et al. reported draft genome sequence of *Deinococcus aerius* strain TR0125, which was initially isolated as an orange-pigmented, non-motile, desiccation-tolerant, UV- and gamma-resistant, and coccoid bacterium in the dust sample collected from the upper troposphere in Japan.

The genome sequence suggested that the strain TR0125 lacks several genes involved in metabolisms of nitrogen, arginine, ornithine and carbohydrate. It is consistent with the biochemical feature of this bacterium. The strain TR0125 exhibited much slower growth than *D. radiodurans*. This feature might be related to the fact that the strain TR0125 genome possesses only one rRNA operon, contrasting many other *Deinococcus* bacteria, which have multiple rRNA operons (Table 1). Whereas, genes related to radiation/desiccation resistance such as *pprI*, *pprA*, *recA*, *ddrA*, and *ddrO*, which are characteristic of *Deinococcus* bacterial spicies, are also conserved in the strain TR0125. Accumulating genome sequence data of *Deinococcus* bacteria will facilitate better understanding of molecular mechanisms relating their highly efficient DNA repair ability.

Discovery of another organism hyper resistant to DNA double-strand breaks [2]

Every living organism has DNA, which holds information in its chemical structure for building and maintaining an organism, in their cells. Therefore, protecting DNA from various types of damage is highly critical for all living organisms. Among various types of DNA damage, double-strand breaks (DSBs) are most serious and difficult to repair exactly. *Deinococcus* bacteria demonstrated above have superior ability in repairing DSBs effectively, but there are several other organisms that also show strong resistance to DSBs. According to the recent report by Yokota et al., the moss *Physcomitrella patens* seems to be eligible to join the group of organisms that are hyper resistant to DSBs (Fig.2). The γ-irradiation experiments revealed that *P. patens* single cells (protoplasts) were 200-times more radioresistant than human single cells. Subsequently, DSB yield measurement by a pulsed-field gel electrophoresis assay indicated that the DSB yield in *P. patens* was half to one-third of those in mammals and yeasts. Furthermore, the DSB yield per cell per 50% lethal dose in *P. patens* was three- to six-times higher than those in mammals and yeasts. The series of findings suggested that DSB induction is inhibited in *P. patens* cells and that they can survive even when a large number of DSBs are induced. Revealing molecular mechanisms of the DSB resistance in *P. patens* is a challenge of the future.

References

[1] K. Satoh *et al.*, Genome Announc., **6**, e00080-18 (2018).
[2] Y. Yokota *et al.*, Genes, **9**, 76 (2018).
[3] Y. Yang *et al.*, Int. J. Syst. Evol. Microbiol., **59**,1862 (2009).

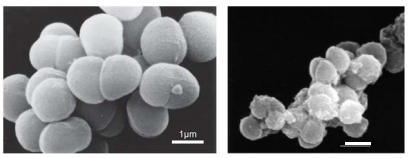

Fig. 1. Scanning electron micrographs of *D. radiodurans* (left) and *D. aerius* (right). Bars indicate 1 μm. The photograph for *D. aerius* is from [3] with modification to highlight the size bar.

Table 1
Comparison of *Deinococcus* genome sequences.

	D. radiodurans	*D. grandis*	*D. aerius*
No. of nucleotides determined	4,092,497	3,344,765	**4,524,446**
% GC	66.3	66.5	**68.0**
No. of genes	3,079	4,043	**4,446**
No. of tRNAs	50	51	**52**
No. of rRNAs (5S/16S/23S)	3/3/3	4/4/4	**1/1/1**

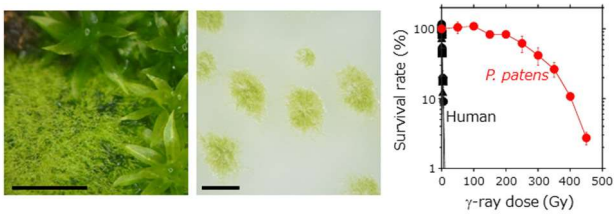

Fig. 2. Clonogenic ability of *P. patens* (left) was measured by a colony formation assay (centre). Bars indicate 2 mm. *P. patens* cells were 200-times more radioresistant than human cells (right).