SEARCH FOR RADIORESISTANCE

THE UNLIMITED POSSIBILITIES OF RADIORESISTANT *E. coli*

- **Botchan Lab.** Tokyo
- **iGEM** team from **TUS**
- **Tokyo University of Science**

ボッチャン ラボトーキョー

A lot of tanks for contaminated water
(Photo credit: Tokyo Electric Power Company Holdings)

Introduction

In 2011, Great East Japan Earthquake causes the nuclear accident in Fukushima Daiichi Nuclear Power Station. People have made efforts to solve contamination problems since then, but still had tons of contaminated water, soils and biomass. We think that these problems could be solved by **BIOREMEDIATION**.

Visiting National Institute of Genetics

When we visited National Institute of Genetics last December, Mr. Andachi told us that we need to consider the effect of uptake radioactive materials on bacteria in addition to removal technologies. Since the 2011 iGEM team SYSU-China searched cesium uptake, we focused on radiation-resistance.

Search for radiation-resistance

We have been searching for effective genetic combinations which make *Escherichia coli* resistant to radiation. As a first step, we chose **three genes** that are related to high DNA repair ability and oxidative stress tolerance from *Deinococcus radiodurans*. We're trying to evaluate the efficacy of each gene against radiation by comparing postradiation survival rate.

About *recA*

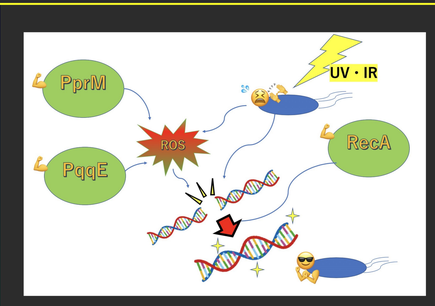
RecA protein promote strand exchange of long DNA for homologous recombination, and are thus involved in repair and reorganization of DNA. Homologous recombination is an essential cellular process in the faithful repair of DNA damage.

About *pprM*

Recent studies show that its RNA-binding domain is necessary for PprM functions to adapt to the extreme environmental stress(e.g. mitomycin C,UV radiation and H_2O_2).

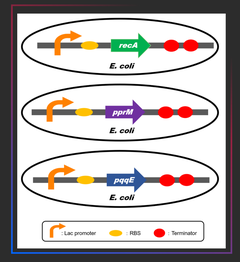
About *pqqE*

When the *pqqE* is expressed in *E. coli*, PQQ synthase is synthesized, and it synthesizes pyrroloquinoline quinone (PQQ). PQQ interact with YfgL and active it to help it exert its ROS removal function.



Materials and Methods

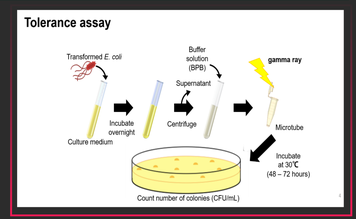
1) Cloning of radioresistance genes from *D.radiodurans*



We try to introduce three genes from *D.radiodurans* —*recA*, *pprM*, and *pqqE*—into *E. coli* DH5α strain. We obtain these three genes by using the colony PCR method, and try to introduce them into *E. coli* by using the In-Fusion cloning technology.

2) Gamma-irradiation and cell survival assay

After gene is introduced, we irradiate gamma ray to our transformant *E. coli* owing *recA*, *pprM*, or *pqqE*, and measure the cell viability.We used *E. coli* containing pSB1C3-RFP vector (BBa_J04450) as a negative control and *D. radiodurans* as a positive control.

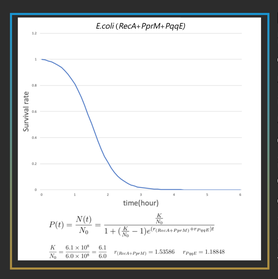


$$\text{Viability} = \frac{N_0}{N}$$

N : the average Colony Forming Unit (CFU/mL) of irradiated samples
N_0 : the average CFU/mL of non-irradiated samples.

Modeling

2D Model

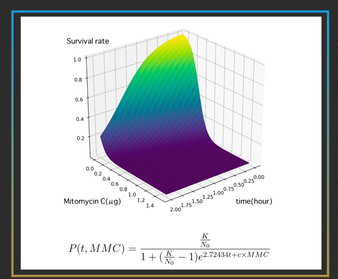


$$P(t) = \frac{N(t)}{N_0} = \frac{\frac{K}{N_0}}{1 + (\frac{K}{N_0} - 1)e^{(r_{(RecA+PprM)} + r_{PqqE})t}}$$

$\frac{K}{N_0} = \frac{6.1 \times 10^8}{6.0 \times 10^8} = \frac{6.1}{6.0}$ $r_{(RecA+PprM)} = 1.53566$ $r_{PqqE} = 1.18848$

In modeling, we modeled how the survival rate changes when you put in uncertain genes. The mechanism was understood by estimating the parameters of the differential equation. As a method, nonlinear regression analysis was performed by drawing the curve using experimental values and literature values.

3D Model

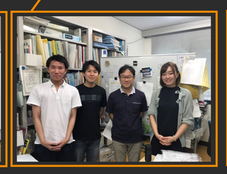


$$P(t, MMC) = \frac{K}{1 + (\frac{K}{N_0} - 1)e^{2.7243Mt + cxMMC}}$$

The survival curve which includes two-variable function can be simulated by using Python's 3DGraph module. (Mitomycin C is used when performing resistance check.) Therefore, we can use not only data by genes but Chemical substances.

Integrated Human Practices

Visiting Pro.Motohashi



Science school for young children

Visiting EARTH SOLUTION INC.

Giving lecture about synthetic biology at local high school

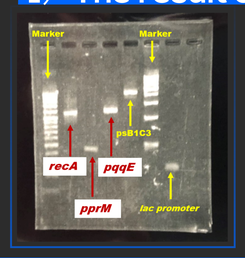
Participated Gifu meet up and iGEM Japan Summer Meet Up

We visited Mr. Keiichiro Kawahara, representatives of EARTH SOLUTION INC., and changed the plan to remove radioactive substances on a small scale and closed system to complement existing technology from the viewpoint of business. If we can establish more effective way to remove radioactive substances and decrease the waste in contaminated area, it may mitigate some problems on radioactive contamination.

Result

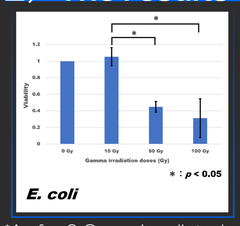
- We obtained *recA*, *pprM*, and *pqqE* genes.
- *E. coli* DH5α (containing BBa_J04450 vector) as negative control showed sensitivity against gamma irradiation.
- *D. radiodurans* as positive control showed resistance against gamma irradiation.
- The transformant *E. coli* harboring *D. radiodurans* 's genes would be more resistant than negative control against gamma irradiation (as a future plan).

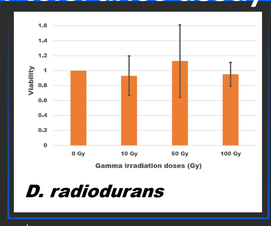
1) The result of cloning



We obtained *recA*, *pprM*, and *pqqE* genes from *D. radiodurans*. We are going to introduce them into *E. coli* by using by using the In-Fusion cloning method.

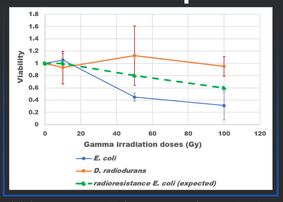
2) The results of tolerance assay



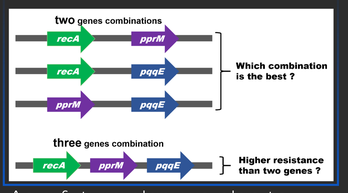


*As for 0 Gy, an irradiated sample was as same as a non-irradiated group, so the survival rate was calculated as always 1.

3) Future plan



We expect that our bacteria would be more resistant than negative control (*E. coli*) and less resistant than positive control (*D. radiodurans*).

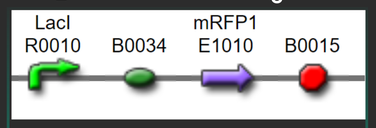


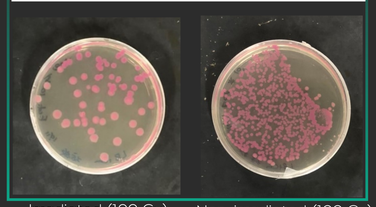
As a future plan, we plan to verify which combination is the best to improve the radiation resistance of *E. coli*.

Parts

Characterization (for the bronze medal criteria)

BBa_J04450 : RFP coding device



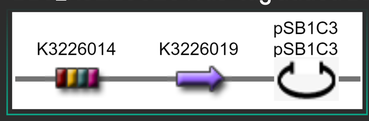


Irradiated (100 Gy) Non-irradiated (100 Gy)

We measured the radiation resistance (gamma ray) of *E. coli* DH5α containing this parts with pSB1C3, and also verified the expression of RFP. The *E. coli* showed sensitivity to radiation, but the expression of RFP seemed normal.
*This result was also used as negative control for the tolerance assay.

Validated parts (for the silver medal criteria)

BBa_K3226020 : His-tag added BBa_K602008.



This part will be an improved part of the device for expressing RecA made by Osaka (2011). His-tags will be added by using PCR (as a primer sequence) so that the expressed RecA could be purified and recovered.

References

• Slade D, Lindner AB, Paul G, Radman M (2009) Recombination and replication in DNA repair of heavily irradiated Deinococcus radiodurans. Cell 136: 1044–1055.

• Zahradka K, Slade D, Bailone A, Sommer S, Averbeck D, et al. (2006) Reassembly of shattered chromosomes in Deinococcus radiodurans. Na- ture 443: 569–573. • Li W, Ma Y, Yang J, Xiao F, Wang W, He S. Indian J Microbiol. 2017 Dec; 57(4):492-498. Epub 2017 Oct 23. • Khairnar NP, Kamble VA, Mangoli SH, Apte SK, Misra HS. Mol Microbiol. 2007 Jul;65(2):294-304. • Inactivation of Escherichia coli JM109, DH5alpha, and O157:H7 suspended in Butterfield's Phosphate Buffer by gamma irradiation., Sommers CH, Rajkowski KT., Journal of Food Science, 2008 Mar;73(2):M87-90.

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