

Radiation irradiation experiment

Lab notes

0918

1. Making culture medium TGY broth and LB+Cm broth
2. preparation BPB

0919

1. Making culture medium LB+Cm plate and TGY plate
2. Pre-culture (*D.radiodurans* / *E.coli*)

0920

1. Preparation of gamma-irradiation samples
Prepare samples for unirradiated /10 Gy /50 Gy for each bacteria
2. Gamma irradiation
3. Plate samples

0923

1. Making culture medium LB+Cm plate and TGY plate

0925

1. Pre-culture (*D.radiodurans* / *E.coli*)

0926

1. Preparation of gamma-irradiation samples
Prepare samples for unirradiated /5 Gy /10 Gy /50 Gy for each bacteria
2. Gamma irradiation
3. Plate samples
4. Pre-culture (*D.radiodurans* / *E.coli*)

Result

<i>E.coli</i>		Dilution rate		
		10 ⁰	10 ⁵	10 ⁶
Irradiation amount (Gy)	unirradiated	-	379	120
		-	507	128
	5 Gy	Alive (Liquid)	0	106
		-	276	134
	10 Gy	Alive	724	200
		-	416	47
	50 Gy	Not alive	117	51
		-	280	91

<i>D.rad</i>		Dilution rate		
		10 ⁰	10 ⁴	10 ⁵
Irradiation amount (Gy)	unirradiated	-	263	46
		-	389	39
	5 Gy	Alive (Liquid)	0	54
		-	52	43
	10 Gy	Alive	422	10
		-	524	0
	50 Gy	0	0	0
		-	0	0

Incubation time : About 90 hours

There were small colonies of *D.radiodurans*. *D.radiodurans* irradiated with 50 Gy was not alive. There was a possibility of sowing *E.coli* on the TGY plates. (The *E.coli* colony was observed.) We decided to do the experiment of irradiating *D.radiodurans* with 50 Gy again. *D.radiodurans* plates were all contaminated with *E.coli*. We also decided to stop the 5 Gy irradiation anymore.

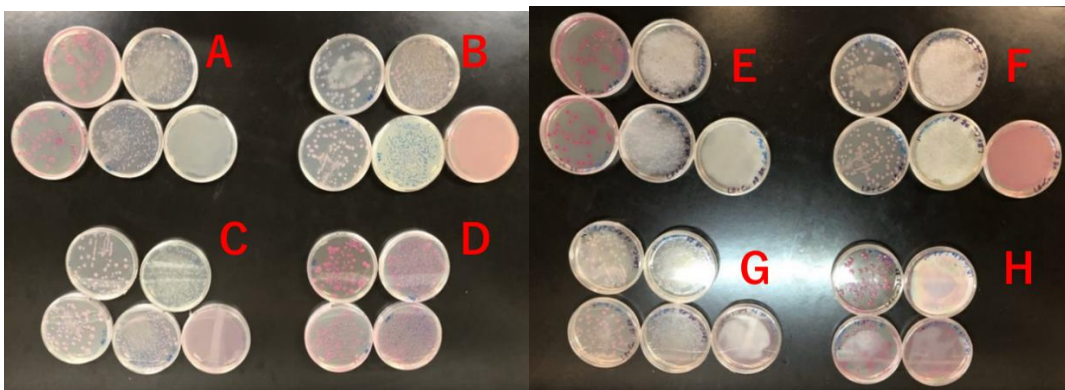


Fig.1 *E.coli* (A:50 Gy B:10 Gy C:5 Gy D:unirradiated) Back

Fig.2 *E.coli* (E:50 Gy F:10 Gy G:5 Gy H:unirradiated) Front

**E.coli*'s colonies showed red purple, this is because the cell expressed RFP gene included in vector (pSB1C3). On the other hand, *D.radiodurans* colonies showed orange as wild type.

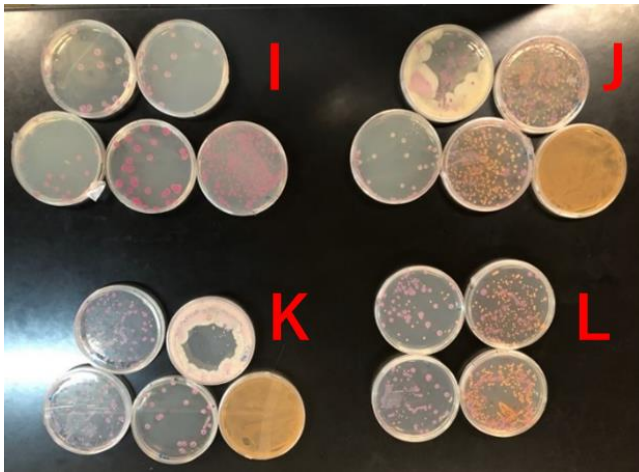


Fig.3 *D.radiodurans* (I:50 Gy J:10 Gy K:5 Gy L:unirradiated) Back

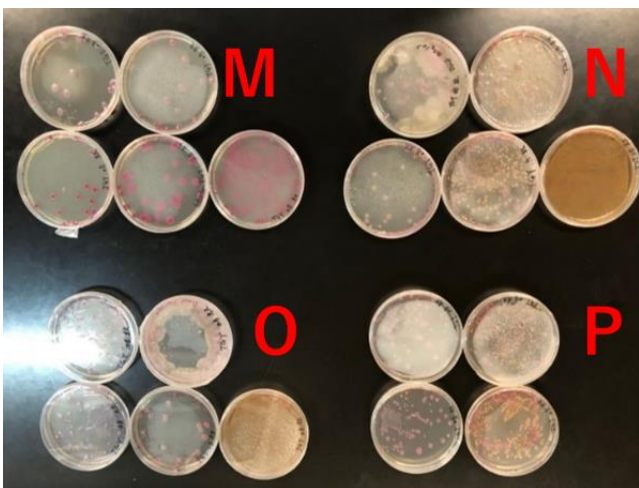


Fig.4 *D.radiodurans* (M:50 Gy N:10 Gy O:5 Gy P:unirradiated) Front

0927

1.Preparation of gamma-irradiation samples

Prepare samples for unirradiated /100 Gy for each bacteria

2. Gamma irradiation

3. Plate samples

Result

<i>E.coli</i>		Dilution rate		
		10 ⁰	10 ⁵	10 ⁶
Irradiation amount (Gy)	unirradiated	-	665	95
		-	463	Can't count (Liquid)
100 Gy	Alive (Liquid)		132	134
		-	110	35

<i>D.rad</i>		Dilution rate		
		10 ⁰	10 ⁴	10 ⁵
Irradiation amount (Gy)	unirradiated	-	93	25
		-	112	8
100 Gy	Alive (Liquid)		181	14
		-	204	9

Incubation time: About 60 hours

All *D.radiodurans* plates were contaminated with *E.coli* again.

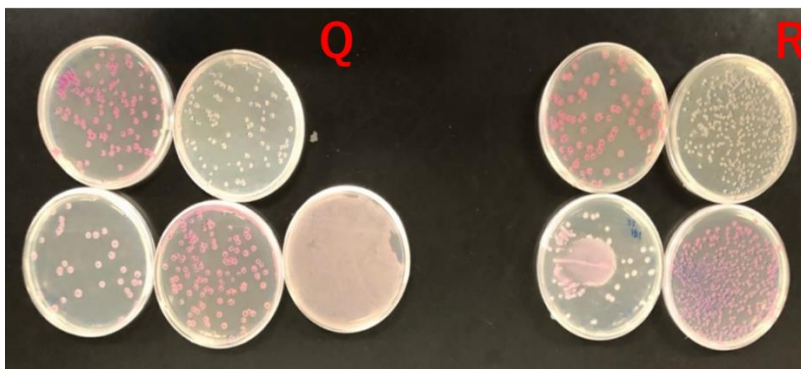


Fig.5 *E.coli* (Q:100 Gy R:unirradiated) Back

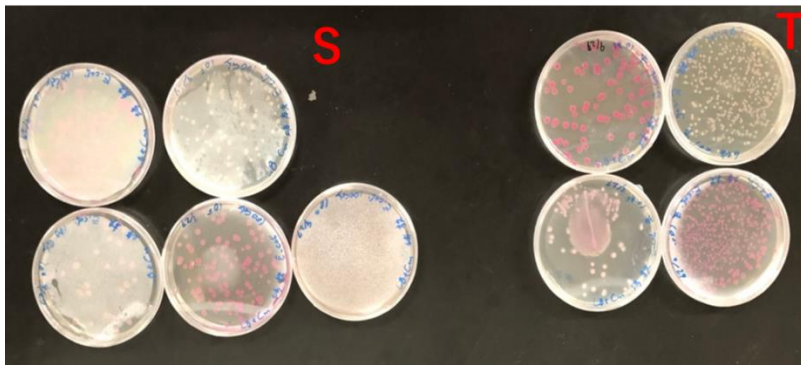


Fig.6 *E.coli* (S:100 Gy T:unirradiated)

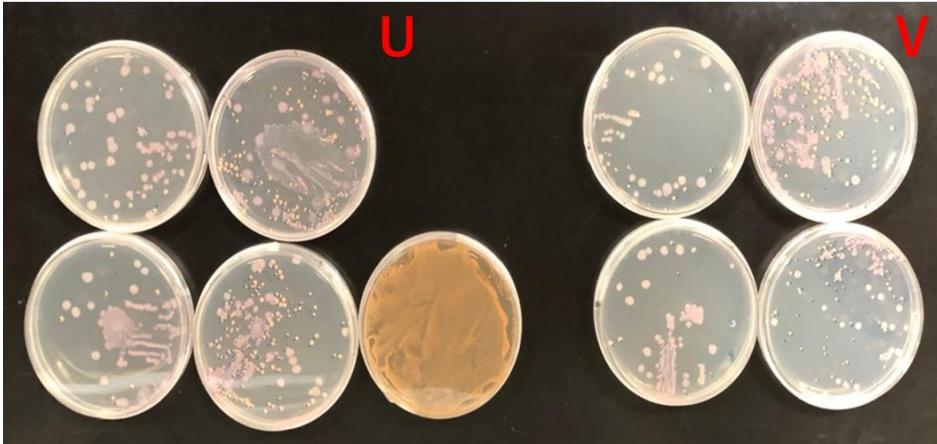


Fig.7 *D.radiodurans* (U:100 Gy V:unirradiated) Back

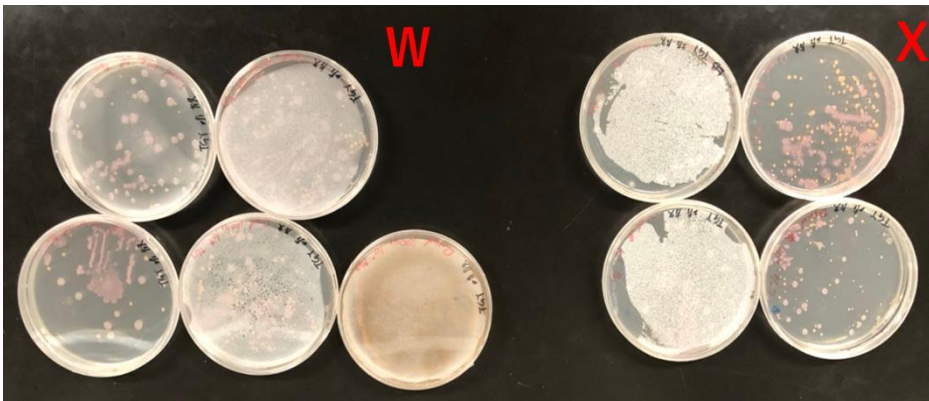


Fig.8 *D.radiodurans* (W:100 Gy X:unirradiated) Front

0930

1. Pre-culture (*D.radiodurans* / *E.coli*)

1001

1.Preparation of gamma-irradiation samples

Prepare samples for unirradiated /100 Gy for each bacteria

2. Gamma irradiation

3. Plate samples

Result

<i>E.coli</i>		Dilution rate		
		10 ⁰	10 ⁵	10 ⁶
Irradiation amount (Gy)	unirradiated	-	520	81
		-	519	24
		-	316	53
100 Gy	Alive (Liquid)	36	7	
	-	25	7	

<i>D.rad</i>		Dilution rate		
		10 ⁰	10 ⁴	10 ⁵
Irradiation amount (Gy)	unirradiated	-	470	87
		-	430	72
		-	-	-
100 Gy	Alive (Liquid)	403	28	
	-	401	81	

Incubation time (*E.coli*): About 44 hours

Incubation time (*D.radiodurans*): About 69 hours

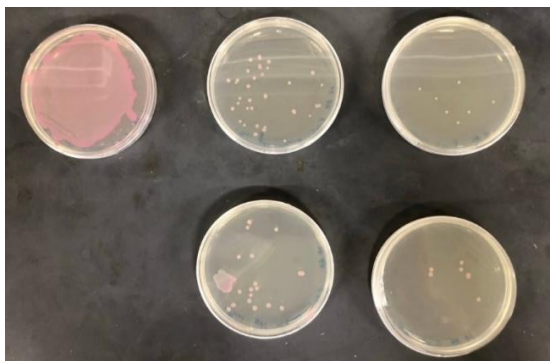


Fig. 9 *E.coli* (100 Gy) Back

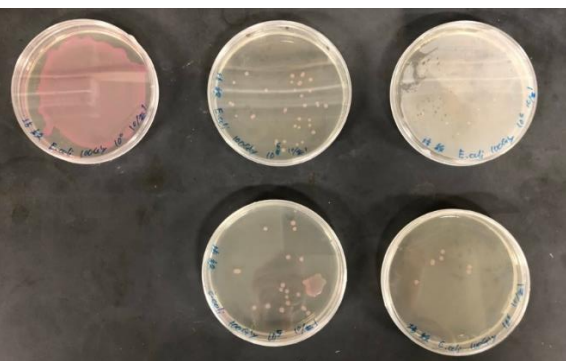


Fig. 10 *E.coli* (100 Gy) Front

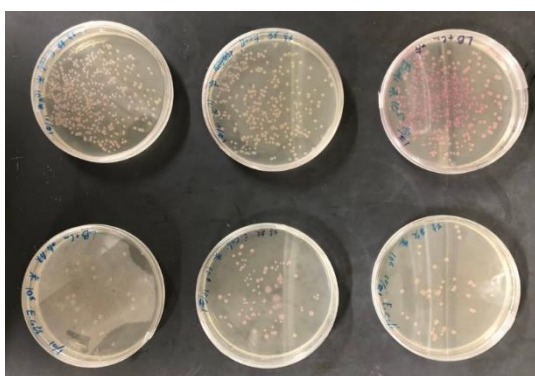


Fig. 11 *E.coli* (unirradiated) Front

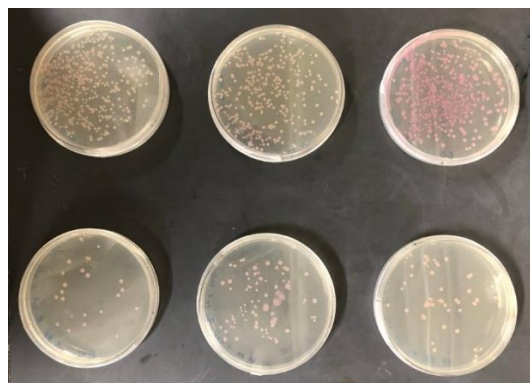


Fig. 12 *E.coli* (unirradiated) Back

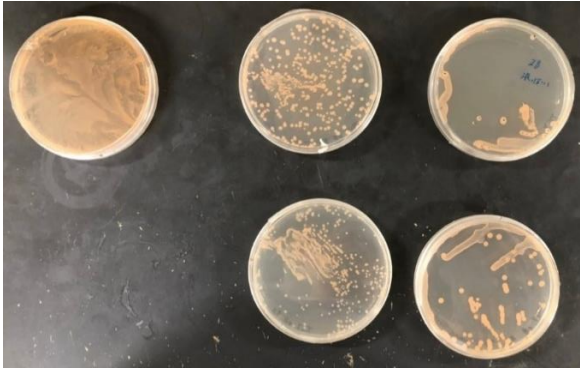


Fig. 13 *D.radiodurans* (100 Gy) Back

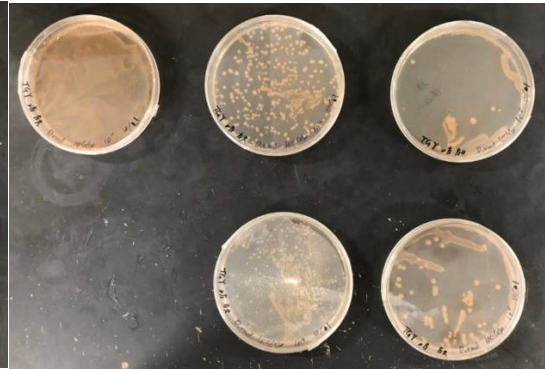


Fig. 14 *D.radiodurans* (100 Gy) Front

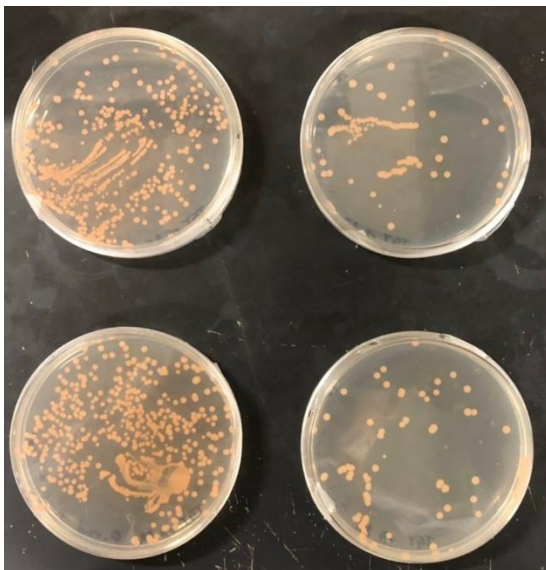


Fig. 15 *D.radiodurans* (unirradiated) Back

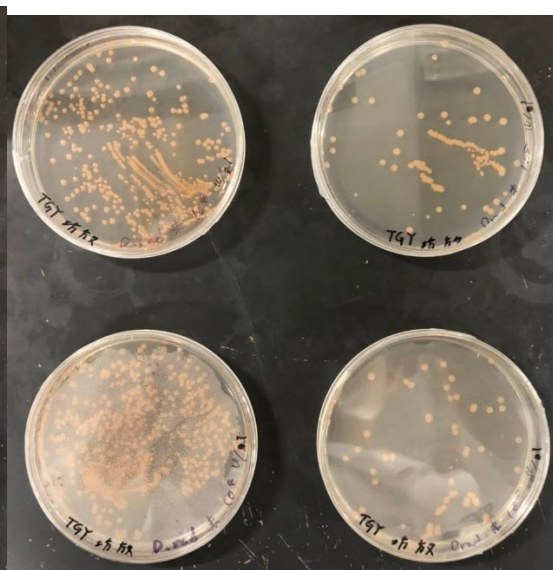


Fig. 16 *D.radiodurans* (unirradiated) Front

1003

1. Pre-culture (*D.radiodurans* / *E.coli*)

1004

1. Preparation of gamma-irradiation samples

Prepare samples for unirradiated /10 Gy /50 Gy for each bacteria

2. Gamma irradiation

3. Plate samples

Result

		Dilution rate		
		10 ⁰	10 ⁵	10 ⁶
Irradiation amount (Gy)	unirradiated	-	398	36
		-	270	44
	10 Gy	Alive (Liquid)	445	-
		-	420	-
	50 Gy	Alive (Liquid)	139	19
		-	112	16

		Dilution rate		
		10 ⁰	10 ⁴	10 ⁵
Irradiation amount (Gy)	unirradiated	-	248	38
		-	163	65
	10 Gy	Alive (Liquid)	516	49
		-	341	53
	50 Gy	Alive (Liquid)	180	4
		-	313	51
	unirradiated 2	-	133	22
		-	90	17
	50 Gy 2	Alive (Liquid)	211	48
		-	169	25

Contamination was seen on several plates of *E.coli*.

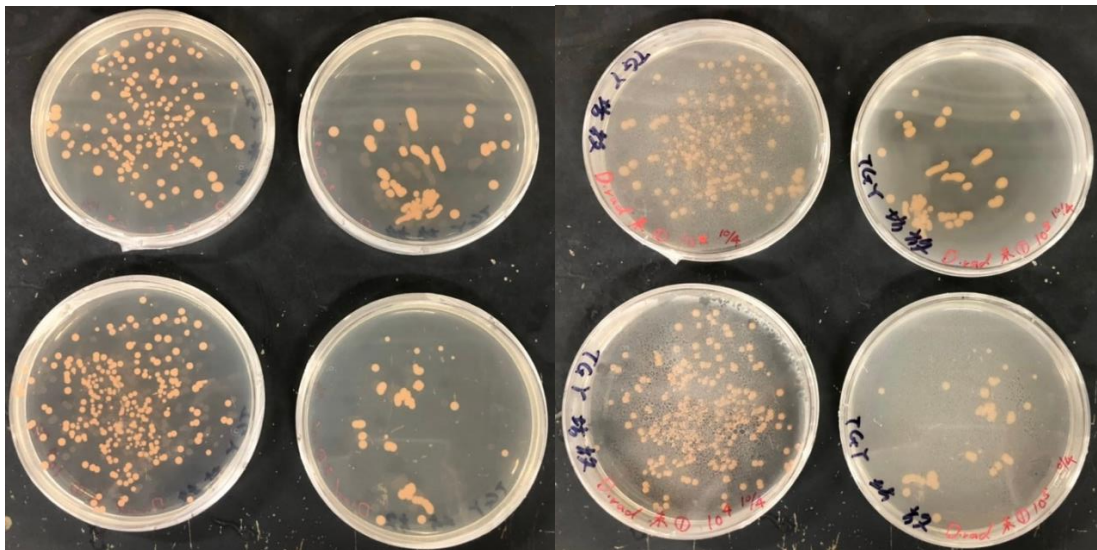


Fig.17 *D.radiodurans* (unirradiated 1) Back Fig. 18 *D.radiodurans* (unirradiated 1) Front

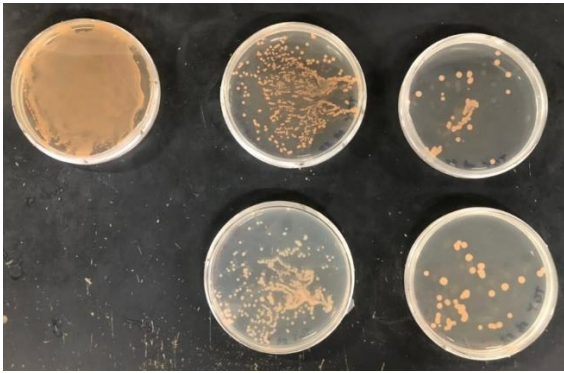


Fig. 19 *D.radiodurans* (10 Gy) Back

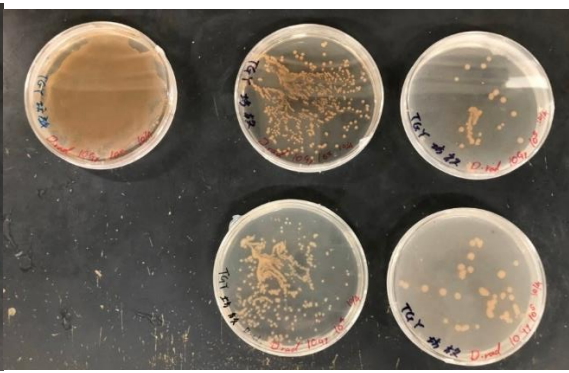


Fig. 20 *D.radiodurans* (10 Gy) Front

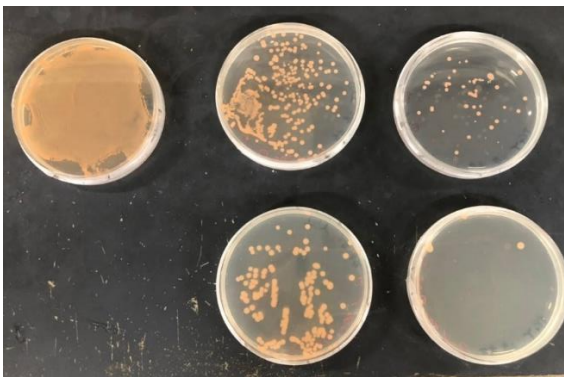


Fig. 21 *D.radiodurans* (50 Gy 1) Back

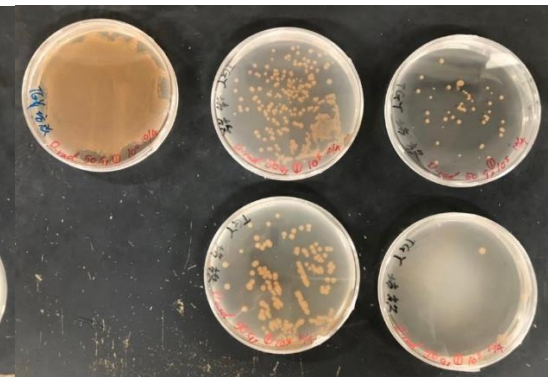


Fig. 22 *D.radiodurans* (50 Gy 1) Front

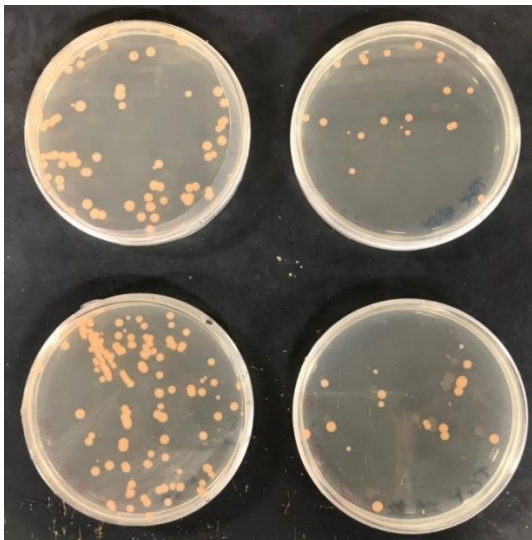


Fig.23 *D.radiodurans* (unirradiated 2) Back

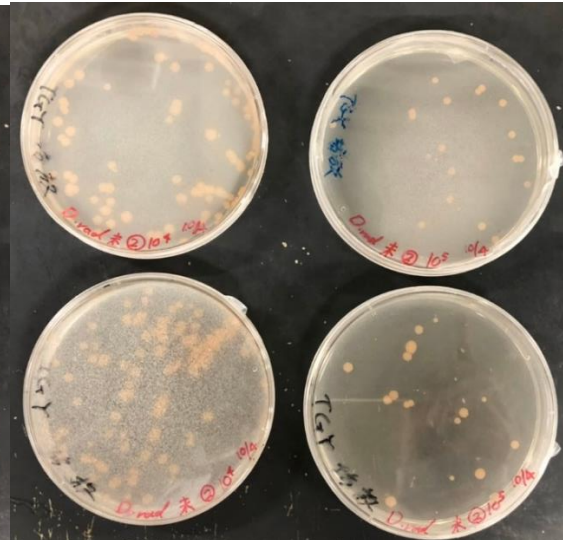


Fig. 24 *D.radiodurans* (unirradiated 2) Front

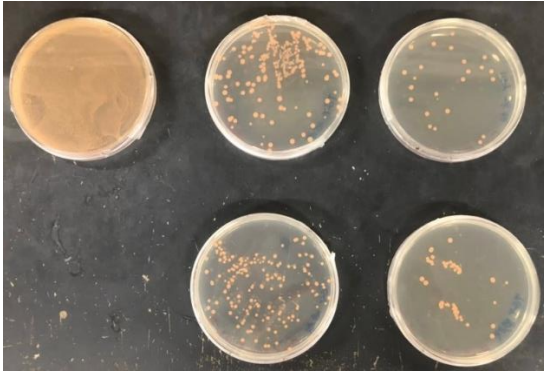


Fig. 25 *D.radiodurans* (50 Gy 2) Back

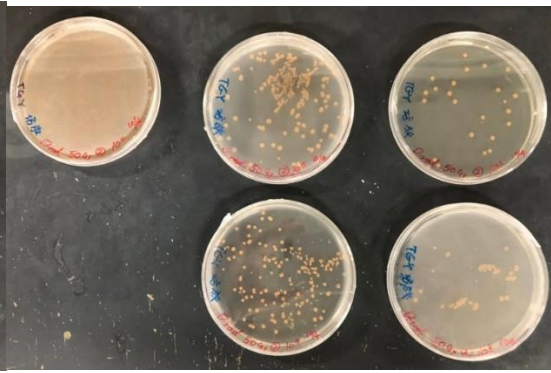


Fig. 26 *D.radiodurans* (50 Gy 2) Front

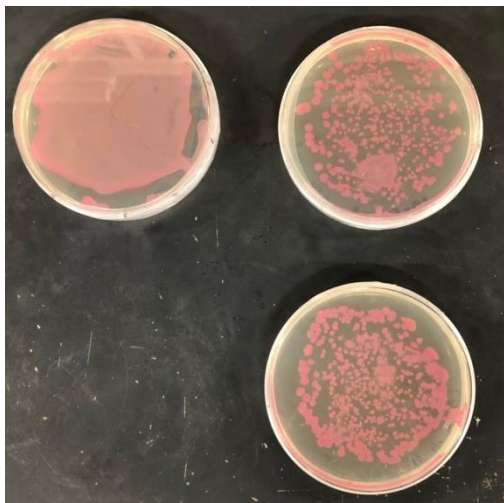


Fig. 27 *E.coli* (10 Gy) Back

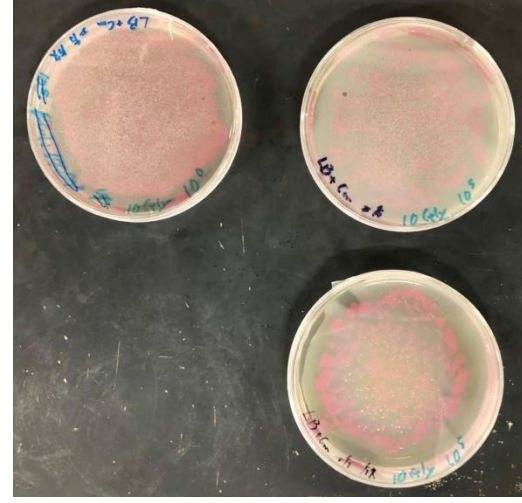


Fig. 28 *E.coli* (10 Gy) Front

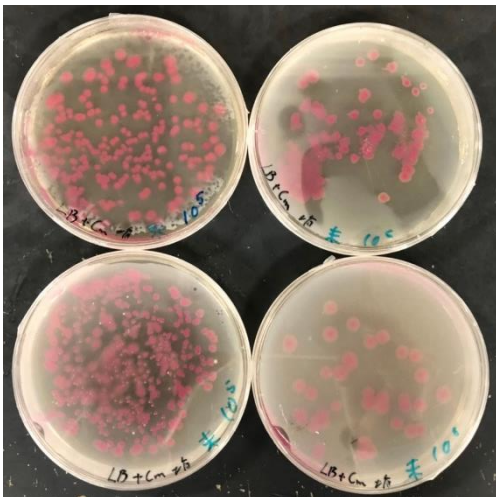


Fig. 29 *E. coli* (unirradiated) Front

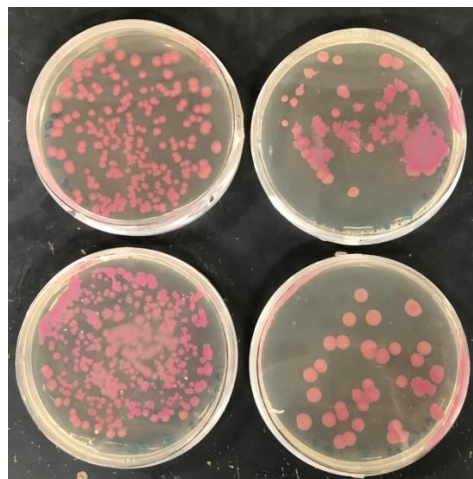


Fig. *E. coli* 30 (unirradiated) Back

1007

1. Pre-culture (*D.radiodurans* / *E.coli*)

1008

1. Preparation of gamma-irradiation samples

Prepare samples for unirradiated /10 Gy /50 Gy for each bacteria

2. Gamma irradiation

3. Plate samples

Result

<i>E.coli</i>		Dilution rate		
		10 ⁰	10 ⁵	10 ⁶
Irradiation amount (Gy)	unirradiated	-	621	71
		-	457	80
	10 Gy	Alive (Liquid)	579	81
		-	474	50
	50 Gy	Alive (Liquid)	248	33
		-	242	21

<i>D.rad</i>		Dilution rate		
		10 ⁰	10 ³	10 ⁴
Irradiation amount (Gy)	unirradiated	-	526	96
		-	518	61
	10 Gy	Alive (Liquid)	108	90
		-	381	41
	50 Gy	Alive (Liquid)	536	54
		-	378	47

Incubation time: About 45 hours

Contamination was seen on several plates of *E.coli*. *D.radiodurans* grew poorly in pre-culture. Therefore, we changed dilution ratio to 10³ and 10⁴ for *D.radiodurans*.

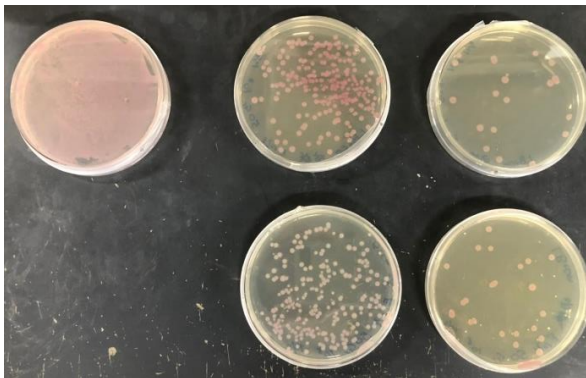


Fig. 31 *E.coli* (50 Gy) Back

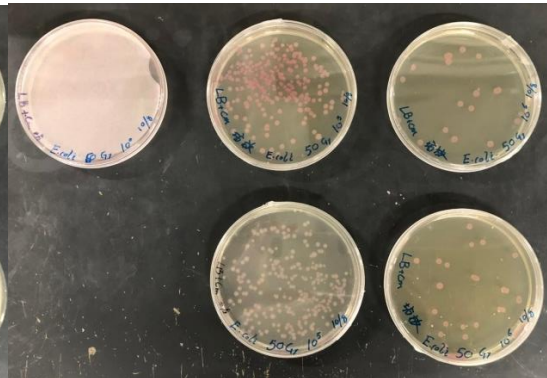


Fig.32 *E.coli* (50 Gy) Front

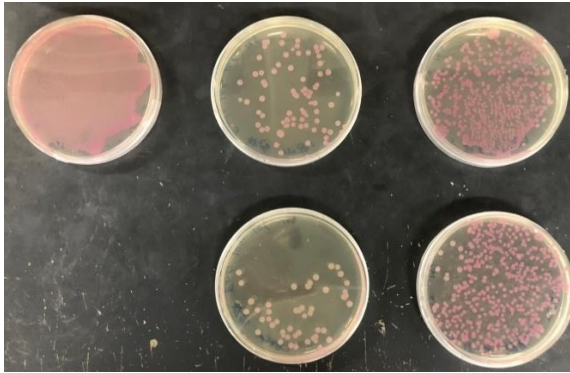


Fig. 33 *E.coli* (10 Gy) Back

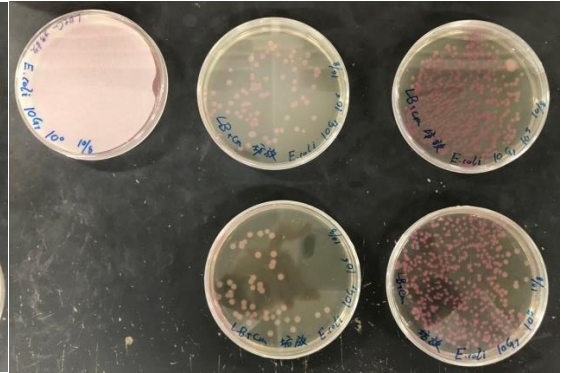


Fig. 34 *E.coli* (10 Gy) Front

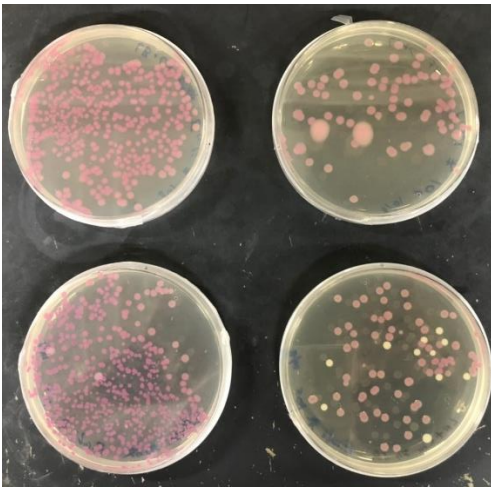


Fig. 35 *E.coli* (unirradiated) Back

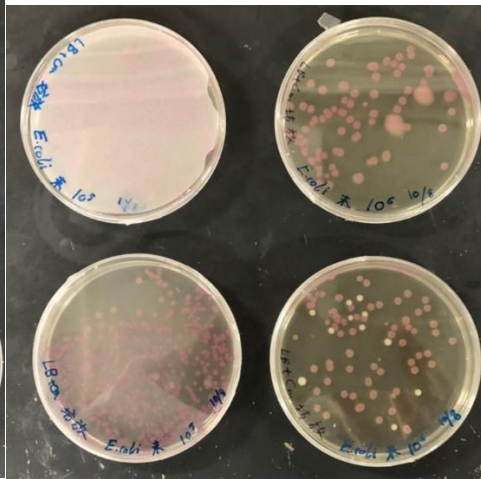


Fig. 36 *E.coli* (unirradiated) Front

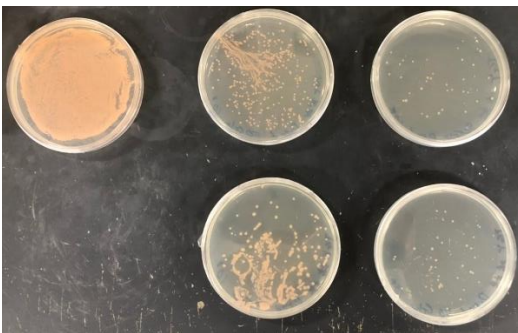


Fig. 37 *D.radiodurans* (50 Gy) Back

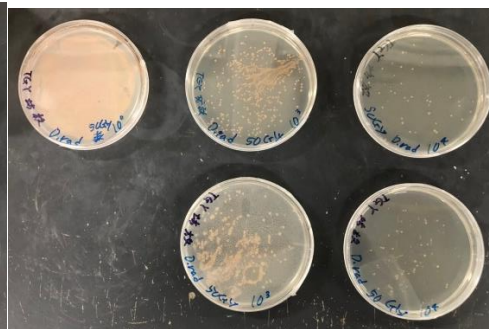


Fig. 38 *D.radiodurans* (50 Gy) Front

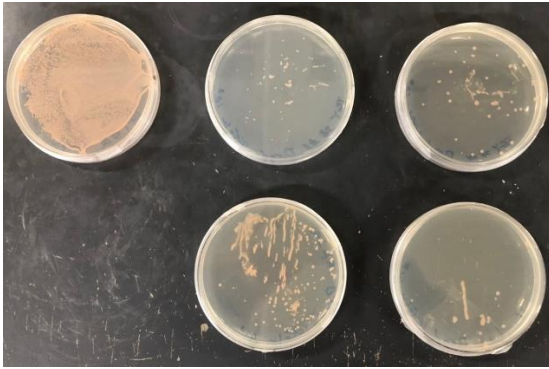


Fig. 39 *D.radiodurans* (10 Gy) Back

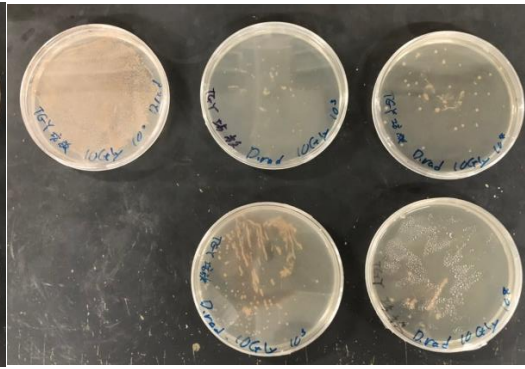


Fig. 40 *D.radiodurans* (10 Gy) Front

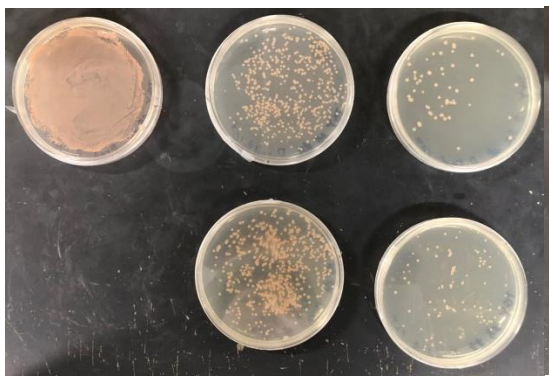


Fig. 41 *D.radiodurans* (unirradiated) Back

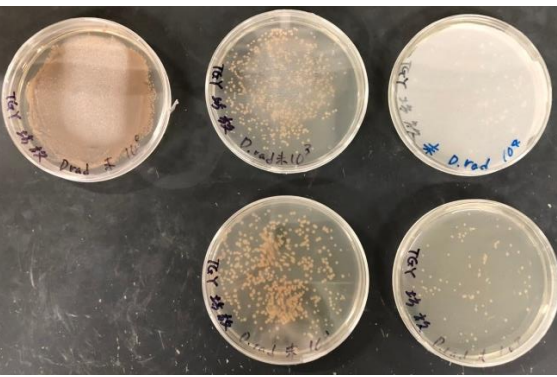


Fig. 42 *D.radiodurans* (unirradiated) Front

1010

1. Pre-culture (*D.radiodurans* / *E.coli*)

1011

1. Preparation of gamma-irradiation samples

Prepare samples for unirradiated /100 Gy for each bacteria

2. Gamma irradiation

3. Plate samples

Result

<i>E.coli</i>		Dilution rate		
		10^0	10^5	10^6
Irradiation amount (Gy)	unirradiated	-	739	84
		-	414	89
	100 Gy	Alive (Liquid)	141	20
-		121	14	
-		83	16	

<i>D.rad</i>		Dilution rate		
		10^0	10^4	10^5
Irradiation amount (Gy)	unirradiated	-	640	82
		-	729	41
	100 Gy	Alive (Liquid)	478	96
-		236	78	

Incubation time: About 66 hours

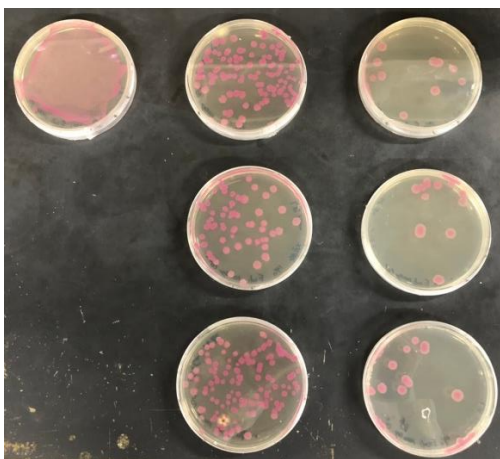


Fig. 43 *E.coli* (100 Gy) Back

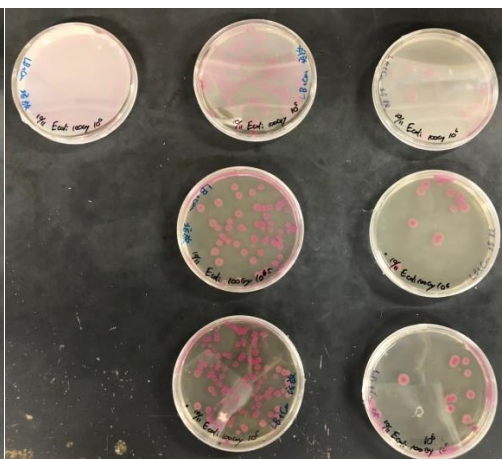


Fig. 44 *E.coli* (100 Gy) Front

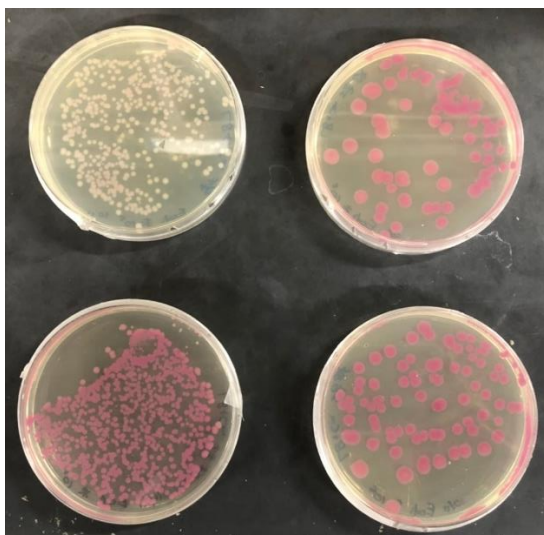


Fig. 45 *E.coli* (unirradiated) Back

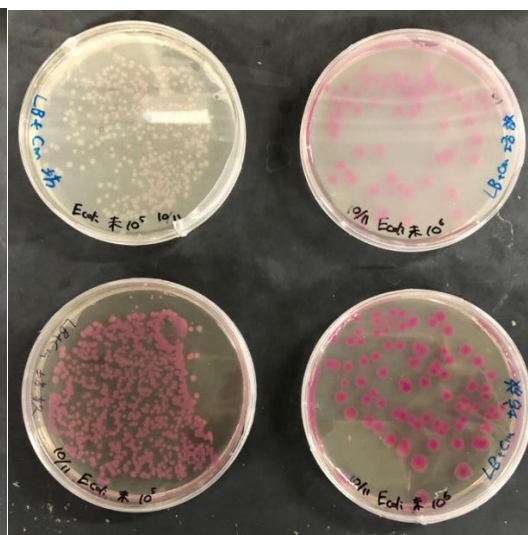


Fig. 46 *E.coli* (unirradiated) Front

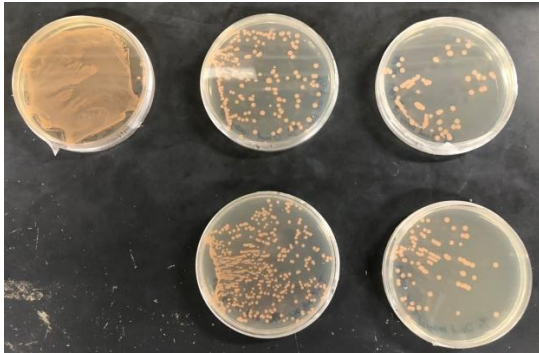


Fig. 47 *D.radiodurans* (100 Gy) Back

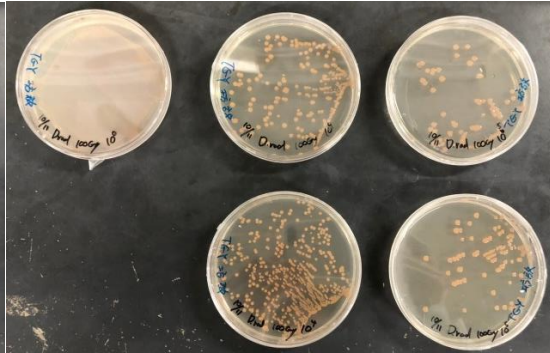


Fig. 48 *D.radiodurans* (100 Gy) Front

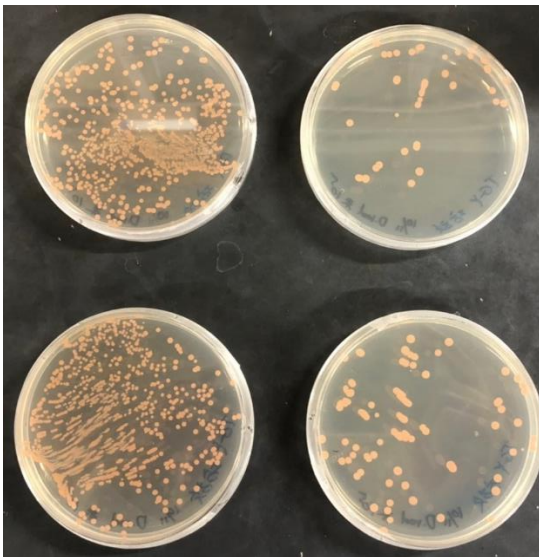


Fig.49 *D.radiodurans* (unirradiated) Back

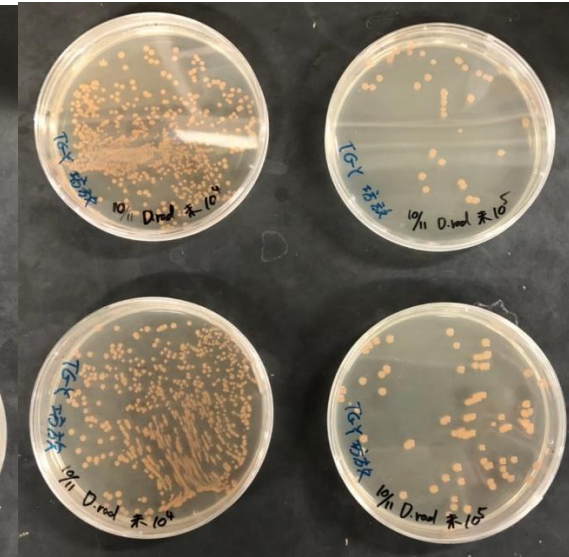


Fig.50 *D.radiodurans* (unirradiated) Front