Dr. Fuentes' lab: Laboratorio de Microbiología y Mutagénesis Ambiental

RADIORESISTANT PROTOCOL: LIVE/DEAD-Baclight™ Bacterial Viability Kit L7012.

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a) Serratia marcescens growth curve

-Liquid medium

1. Take 0.5 mL overnight culture and inoculate in 30 mL of M9Y-Gly medium at 28 ºC and 150 rpm.
2. Take 1 mL of overnight culture every 12 hours and measure the optical density at 600nm and 535nm.
3. Identify the time to reach the stationary phase and this growth will be used for essays next.

-Solid medium

4. Take 0.5 mL overnight culture and spread over agar M9Y-Gly medium at 28 ºC.
5. Every 12 hours, aggregate sterile distilled water (10 mL) to plates and detach the bacterial cells.
6. Take 1 mL of bacterial suspension and measure the optical density at 600nm and 535nm.

b) Dose-response curve and cell survival through CFU (Prada-medina et al. 2016)

7. Take 7 mL of culture in stationary phase and centrifugate at 7500 rpm for 10 minutes, remove the supernatant and resuspend the pellet in 7 mL of 0.85% NaCl.
8. Distribute into Petri dishes with a 9 cm diameter and irradiate in darkness using a UVA (320-400 nm)/UVB (280-315 nm) irradiation chamber from Opsytec Dr. Grobel, Ettlingen, Baden-Wurttemberg, Germany.

A single log reduction is a 90% reduction of organisms (Light Sources Inc. 2014).

9. The UVB dose range will be between a single log reduction (LD_{10}) and LD_{50} (24.2 – 48.4 J/m^2) (Light Sources Inc. 2014)
10. Irradiated and no-irradiated cells will be diluted in 900 µL of 0.85 % NaCl and plated on M9Y-Gly media during 48h of incubation at 28 ºC in the dark.
11. Colony-forming units will be counted and bacteria concentration will be determinate. The results are expressed as survival percentages.

c) Dose-response curve and cell count in Neubauer chamber.

1. Add 1 mL from suspension irradiated (step 6) to 20 mL of 0.85% NaCl (for live bacteria) and suspension no-irradiated of 20 mL of 70% isopropyl alcohol (for killed bacteria).
2. Incubate both samples at room temperature for 1 hour, mixing every 15 minutes.
3. Obtain pellet both samples by centrifugation aforementioned conditions.
4. Resuspend both pellets in 20 mL of 0.85% NaCl and centrifuge again as in step 3.
5. Resuspend both pellets in separate tubes with 10 mL of 0.85% NaCl.
6. Mix 6 µL of component A (SYTO® 9) with 6 µL of component B (Propidium Iodide) in a microfuge tube and with mix well.

7. Prepare a 2X stain solution by adding the entire 12 µL of the above mixture to 2 mL of filter-sterilized distilled water (dH_2O) in a glass culture tube and mix well.
8. Pipet 100 µL of each of the bacterial cell suspension (from step 1 of section b) and mix with 100 µL of the 2X staining solution (from step 7 of section b) to each well and mix thoroughly by pipetting up and down several times
9. Incubate at room temperature in the dark for 15 minutes.
10. Pipette 30 µL of the stained bacterial suspension (from step 8 of section b) and count live and dead cells with a Neubauer chamber in an inverted motorized microscope Axio Observer 7 model.
11. Determine the mean lethal dose using the graphical method (Ara et al. 2009). That is cell viability (log % viability) versus the linear function of UVB doses.
12. Using this linear scale, it's can LD_{50} estimate and its confidence levels by interpolation.

d) Recovery time post-irradiation through fluorescence microplate readers (Robertson et al. 2019)

1. The UVB dose will depend on step 5 of section (a).
2. Repeat steps 1 to 6 from section (b) for each irradiated bacterial aliquot taken every hour.
3. Pipet 100 µL of each of the bacterial cell suspension into separate wells of a 96-well flat-bottom microplate in triplicate. The outside wells (rows A and H and columns 1 and 12) are usually kept empty to avoid spurious readings.
4. Using a new tip for each well, pipet 100 µL of the 2X staining solution to each well and mix thoroughly by pipetting up and down several times.
5. Incubate at room temperature in the dark for 15 minutes.
6. Carry the microplate to the inverted motorized microscope Axio Observer 7 model and adjust the fluorescence filters for each dye.
7. Use the equation next with adjusted dye ratio for calculate %live cells:

$$\%live\ cells\ = \frac{(100\ X\ \frac{SYTO\ 9}{PI})}{(1 + \frac{SYTO\ 9}{PI})}$$

1. Plot the fluorescence intensity at 600-610 nm (PI) against % live cells.
2. Analyze each scatters plot with linear regression analysis and generate the best-fit value by the coefficient determination (R).
3. The R-value is given to the wavelength associated with the best modeling of % live cells.

References

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