How inducing microbial activity alters culturability

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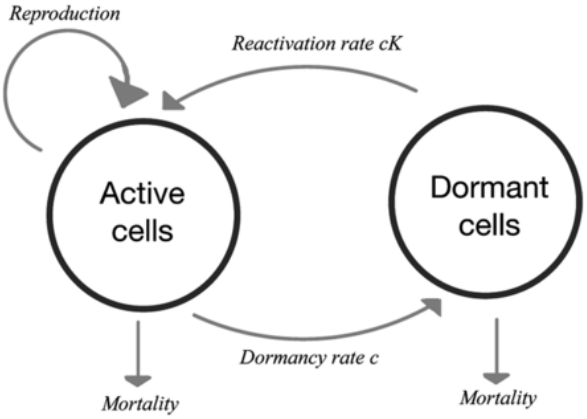
Background:

- The human body is covered with many millions of bacteria.
- Bacteria on our skin can be made to grow (cultured) on agar plates and studied.
- However, culturing bacteria from environments, such as skin, can be challenging because the majority bacterial cells can be dormant.
- Dormancy also allows bacteria to persist in conditions that are too poor for growth.
- Research suggests that 50-60% of skin bacteria are dormant while the rest are active or dead.
- However, dormant bacteria can detect chemical signals that allow them to resuscitate and resume growth in favorable conditions.
- In our laboratory, we study how bacteria resuscitate in the presence of an enzyme called "resuscitation promoting factor" (Rpf). Rpf has the potential to resuscitate many different species of dormant bacteria.

Overarching research question:

- **Does forcing bacteria out of dormancy (inducing activity) alter the ability to culture them?**
- **Considerations: Could forcing bacteria out of dormancy kill them? Will microbes that are forced to become active even grow and reproduce?**



Above: Diagram illustrating the microbial activity-dormancy dynamic where microorganisms can transition in and out of dormancy but only active cells can grow and reproduce.

Terms to know:

- Dormant = A metabolically inactive state. Does not permit growth.

- Enzyme = Biological catalyst that increases the rates of chemical reactions.
- Agar plates = An experimental media used to culture microorganisms.
- Culture = Maintenance of microorganisms in conditions suitable for growth.
- Rpf = Resuscitation promoting factor. An enzyme that resuscitates dormant bacteria by breaking apart peptidoglycan.
- Peptidoglycan = A major component of bacterial cell wall that nearly all bacteria possess.
- Biological signal = A chemical indication of a "good" environment to exit dormancy.
- Resuscitate = Transition from dormancy to activity.

Experimental set up: Six treatments (Five concentrations of Rpf+ and one Rpf- control) x five replicate = 30 petri plates

Timeline of program

Experimental methods (With Venus Kuo):

1. Make nutrient agar plates and physiological saline solution.
2. Extract bacterial community with sterile swab and physiological saline solution.
3. Transfer solution to falcon tube and centrifuge, suspend in saline solution, and divide into five sterile falcon tubes.
4. Add following Rpf concentration in tubes: 0, 0.5, 1.25, 1.7, 2.5, and 5 μmol/L.
5. Incubate skin bacteria with treatment at 30°C for 1 day.
6. Make 10^{-2} and 10^{-4} serial dilutions of skin bacteria and spread plate on nutrient agar.
7. Incubate plates for 2 days at 30°C.
8. Perform colony forming unit counting.

Experimental data analysis (With Ken Locey)

1. Analyze results with statistical software.

Design poster presentation (With Venus and Ken)

Readings provided in email:

- **Lennon_Jones_2011.pdf** – A Nature Reviews Microbiology paper that provides a broad overview for understanding the importance of dormancy for microorganisms.

- **20140331_2216_DoB_Lennon_ProjSumm.pdf** – One-page summary of National Science Foundation Dimensions of Biodiversity Grant outline aims and goals of Lennon research. See also lab website: https://www.indiana.edu/~microbes/