swrA Revertant on Surfactin-coated Plates

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Background

Phase variation in the gene *swrA* controls the chaining state of *B. subtilis*. Many lab strains have a defect in the gene which can increase the amount of chaining and reduce motility.

The bacteria can be encouraged to repair *swrA* when grown on media with low surface tension.

Surfactin Solution

Dissolve 10mg surfactin in 1mL of 20mM NaOH. Maintain sterility.

Plates

Make 0.5-0.8% agar (0.7% works well) LB plates. Supplement as necessary.

Initial selection with surfactin

Treat low-agar plates with surfactin the day you are performing the experiment. Do not store pre-treated.

(1) On a sterilized surface in a laminar flow hood, spot 10uL of surfactin solution onto the center of the plates. Tip plates slightly to spread the surfactin; traditional plating using pipets can tear up the surface of the agar.

(2) 10uL of surfactin will only cover a portion of a plate. Mark the area on the plate that has been coated with surfactin so you'll know where to spot a colony.

(3) Allow the surfactin to dry completely on the plates (takes ~10 min) in the laminar flow hood, right-side up with lids slightly ajar.

(4) Spot cells from a single colony onto the center of the marked surfactin-coated area. Incubate O/N at the appropriate temperature.

Second-day flare on treated plate. Inoculation spot and surfactin region visible at center.

Second selection with surfactin (if necessary)

The following day, the cells will have grown in a sunburst-like flare, expanding out much farther than a spot would on ordinary media. Cells on the edge of this flare should have the

repaired variant of *swrA*.

If the flare has not expanded to cover almost the entire plate, perform a second round of surfactin treatment exactly as before, spotting with cells from the edge of the flare.