***MYXOCOCCUS* SPORE ASSAY**

Adapted from existing protocols by Vinh Pham.
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MATERIALS:

TPM (1.5%, 10mM Tris pH 7.6, 1mM potassium phosphate, 8mM $MgSO_4$)
TPM Agar (1.5% agar, 10mM Tris pH 7.6, 1mM potassium phosphate, 8mM $MgSO_4$)
dH_2O
CTTYE soft agar (1% agar, 1% casitone, 0.2% yeast extract, 10mM Tris pH 7.6, 1mM potassium phosphate, 8mM $MgSO_4$)
CTTYE agar (1.5% agar, 1% casitone, 0.2% yeast extract, 10mM Tris pH 7.6, 1mM potassium phosphate, 8mM $MgSO_4$)

PROCEDURE:

1. Grow *M. xanthus* cultures to Klett readings of 80-120.
2. Pellet cells in 1ml aliquots for 1'. Triplicate or quadruplicate samples might be desired.
3. Wash with 1ml TPM.
4. Resuspend in 100□l TPM
5. Spot 5 x 20□l onto TPM agar plates.
6. Incubate at 33ºC for at least 3 days.
7. Drop 20□l TPM onto each TPM plate.
8. Scrape cells and fruiting bodies with a spatula.
9. Transfer to a microfuge tube containing 500□l dH_2O.
10. Freeze at -20ºC or -80ºC (or 4ºC if samples will be used in enzyme assay).
11. Sonicate 3 times at 70%, 7X, for 1'. Make sure to add ice to cup sonicator to keep the water in the cup cold.
12. Heat the samples at 50ºC for 1-2 hours.
13. Make serial dilutions from 10^{-2} through 10^{-6} with dH_2O in microfuge tubes.
14. Vortex.
15. Transfer to 6ml culture tubes.
16. Add 3ml CTTYE soft agar to each tube.
17. Vortex.
18. Pour each onto a CTTYE agar plate.
19. Slosh soft agar around to mix and let solidify at RT.
20. Incubate at 33ºC.
21. Count colonies. The wild type 10^{-4} and 10^{-5} plates will usually yield numbers between 30-300.
22. If assaying a mutant constructed by insertional disruption with a suicide vector carrying a selectable marker, streak 10-20 colonies onto CTTYE plates with the appropriate antibiotic to score for the % germination of true mutants.