ISOLATING WILD *MYXOCOCCUS* STRAINS

Adapted from Herbert Irschik (Gesellschaft für Biotechnologische Forschung) protocol by Vinh Pham.
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MATERIALS:

HCM Agar (1.5% agar, 0.7% HEPES pH 7.0, 0.15% $CaCl_2$, 0.15% $MgSO_4$, 0.5% cycloheximide/actidione)

VY/2HM Agar (1.5% agar, 5% baker's yeast, 0.7% HEPES pH 7.0, 8mM $MgSO_4$, 10mM $CaCl_2$, 0.5 g/ml vitamin B12/cobalamine)

CHYE (1% casitone, 0.2% yeast extract, 1mM potassium phosphate, 8mM $MgSO_4$, 0.5 g/ml vitamin B12/cobalamine, 0.7% HEPES pH 7.0)

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

CSMX (0.2% yeast extract, 1% HEPES pH 6.8, 10mM $CaCl_2$, 0.5 g/ml vitamin B12/cobalamine)

PROCEDURE:

1. Obtain soil samples from the first 5cm of soil. Good sampling sites include forests (but not coniferous forests due to the low pH), prairie, soils with organic material, rotting wood. River banks may be a poor source of myxobacteria. Consider that it may be better to have small samples from many sites than large samples from just a few sites.
2. Air dry soil samples for 1-2 weeks at room temperature.
3. Streak a "V" aross an HCM agar plate with a loop inoculated with *E. coli* from a fresh plate.
4. Resuspend approximately 20-100 mg of soil in 50 l of TPM. This turns it into a sort of paste.
5. Add the TPM-resuspended sample to the junction formed by the two arms of the "V".
6. Incubate the plate at 50ºC for 1-2 hours. This kills non-heat resistant "contaminants."
7. Transfer plate to 33ºC. It takes about one week to see the first fruiting bodies. A profusion of actinomycete colonies will inevitably crop up, but they and the myxobacteria appear to stay out of each other's way.
8. Streak fruiting bodies onto VY/2HM agar plates and incubate at 28ºC or 33ºC.
9. Inoculate yellow, yellow-orange, and orange-red cells (these are likely to be *Myxococcus xanthus*) into CHYE or CTTYE medium. Pink cells are likely to be *Myxococcus fulvus* and are best grown in CSMX.