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Phenazine Oxidizer Enrichment and Isolation

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ABSTRACT

This is a protocol for the enrichment of phenazine-1-carboxylic acid oxidizing microbes from a soil sample.

Medium composition

1 Stock solutions

100x Freshwater salts:

- 1.71 M sodium chloride
- 197 mM magnesium chloride
- 68 mM calcium chloride
- 671 mM potassium chloride

1000x Trace Elements:

- 20 mM HCl
- 7.5 mM FeCl₃ 6H₂O
- 480 uM H₃BO₃
- 500 uM MnCl₂ 4H₂O
- 6.8 mM CoCl₂ 6H₂O
- 1 mM NiCl₂ 6H₂O
- 12 uM CuCl₂ 2H₂O
- 500 uM ZnCl₂
- 23 uM Na₂SeO₃
- 150 uM Na₂MoO₄

1000x 13-vitamin solution

- 1 mM MOPS pH 7.2
- 100 µg/mL riboflavin
- 30 µg/mL biotin
- 100 µg/mL thiamine HCl
- 100 µg/mL L-ascorbic acid
- 100 µg/mL d-Ca-pantothenate
- 100 µg/mL folic acid
- 100 µg/mL nicotinic acid
- 100 µg/mL 4-aminobenzoic acid
- 100 µg/mL pyridoxine HCl
- 100 µg/mL lipoic acid
- 100 µg/mL Nicotinamide adenine dinucleotide
- 100 µg/mL thiamine pyrophosphate
- 10 µg/mL cyanocobalamin

2 mM reduced phenazine-1-carboxylic acid (PCA)

- In a sealed Balch tube or serum vial, sparge 2 mM oxidized PCA with 80:20 (vol:vol) H₂/CO₂ over Pd (II) until yellow-green in color

- Stir with a magnetic stirbar or otherwise agitate during sparging and overnight afterwards.

Enrichment medium (anoxic)

- 45 mM sodium bicarbonate buffer (buffer)
- 10 mM ammonium chloride (nitrogen source)
- 1 mM potassium phosphate (phosphorus source)
- 1 mM reduced phenazine-1-carboxylic acid (PCA, electron donor)
- 100 uM sodium acetate (carbon source)
- 50 uM sodium sulfide (sulfur source)
- 1x trace elements solution
- 1x freshwater salts solution
- 1x 13-vitamin solution

Isolation medium (oxic or anoxic, agar plates)

- 1 mM sodium phosphate buffer pH 7
- 10 mM ammonium chloride
- 100 uM sodium sulfate
- 1 mM sodium acetate
- 10 mM sodium nitrate
- 1x freshwater salts solution
- 1x trace elements solution
- 1x 13-vitamin solution
- 1.5 % agar

Sampling

- 2 Collect topsoil by scooping directly with 50 mL conical tube.
- 3 Grind with mortar and pestle
- 4 Pass through 2 mm sieve to remove large rocks and organic matter.

- 5 Transfer to anaerobic chamber

Inoculation of enrichment cultures

- 6 Prepare 96-well plate with 190 μ L of anoxic enrichment medium per well.
- 7 Add 20 mg soil into each well.
- 8 Prepare Balch tubes with 9 mL of anoxic enrichment medium in each.
- 9 Add 1 g soil into each tube and seal.
- 10 For both the 96-well plates and Balch tubes, watch for a color change over the next days. The yellow-green reduced PCA will turn clear if oxidized.
- 11 Passage candidate PCA-oxidizing enrichments via 1:10 dilutions into fresh medium, maintaining anoxic conditions.

Isolation

- 12 Plate enrichment cultures onto LB/Agar and the solid isolation medium.

- 13** Grow both oxically and anoxically in an anaerobic chamber.
- 14** Pick colonies of different morphologies and assay PCA-oxidizing capability in the enrichment medium as a pure culture.
- 15** Select isolates that oxidize PCA in pure culture for further study.