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Phenzine 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 compostion

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 $FeCl_3$ $6H_2O$
- 480 uM H_3BO_3
- 500 uM $MnCl_2$ $4H_2O$
- 6.8 mM $CoCl_2$ $6H_2O$
- 1 mM $NiCl_2$ $6H_2O$
- 12 uM $CuCl_2$ $2H_2O$
- 500 uM $ZnCl_2$
- 23 uM Na_2SeO_3
- 150 uM Na_2MoO_4

1000× 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
- 1× 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
- 1× 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 uL 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.