BANANA GUARD

Protocols

MgL growth experiment

Preparation of Cell free extract (Methionine- γ-lyase)

- Grow culture to O.D.$_{600}$ ~0.5 in (50ml), grow 500ml erlenmeyers
  -WT *P.putida* KT2440
  - *P.putida* MgL mutant (1mM IPTG)
  - *P.putida* MgL mutant (0.1mM IPTG)
  - *P.putida* MgL mutant (no induction)
- Induce with IPTG
- After 3 hours, centrifuge 3.220g, 15min, 4°C
- Discard supernatant and wash pellet with equal part ice-cold 10Mm Tris-HCL (PH7.5)
- Repeat washing step
- Resuspend in ice cold 1ml 10mM Tris-HCL (pH 7.5)
  a. Rupture cells via sonification total duration 3min
  b. 30min on, 30 min off (3x)
  c. Operate at level 5, 25% duty cycle
  d. *Keep eppendorf on ICE
  e. Remove via centrifugation 12,000 rpm, 10min, 4°C

Volatile sulfer compounds (VSC) production b MgL

- Put cell free extract with 5mM L-methionine, 100uM PLP, 100mM Tris HCL (pH8)
- Incubate in 25/30 °C for 48h
- Sample at timepoint 0h, 24h and 48h
- GC/MS

GC/MS procedure to measuring DMDS/DMTS

- Let samples rest to room temperature for 15min with an 85-um Carboxenpolydimethylsiloxane fiber
- Clean fibers by heating it to 250°C before use and after injection
- Injector port 250°C in splitless port
- Inject Samples to a DB-FAP colum (30m by 0.32mm;1um film thickness) at helium rate of 2ml/min^{-1}
- Oven temperature at 40°C for 5 min then increase to 100°C with 5°C/min
- Hold at 100°C for 1min
- Increase to 240°C at rate of 30°C/min then
- Hold at 240°C for 1 min then return to 40°C

Reference:

1. Hanniffy, Sean B., et al. "Heterologous Production of Methionine-γ-Lyase from Brevibacterium linens in Lactococcus lactis and Formation of Volatile Sulfur Compounds." *Applied and environmental microbiology* 75.8 (2009): 2326-2332.