

BANANA

GUARD

Protocols



DAPG Protocols

DAPG production on agar

1. Prepare LB agar plates containing correct antibiotic and IPTG concentration
2. Plate 50ul of O/N culture of the following
 - WT *P.putida* KT2440
 - WT DH5- α
 - P.putida* containing *phl* gene cluster (1mM IPTG)
 - *P.putida* containing *phl* gene cluster(0.1mM IPTG)
 - *P.putida* containing *phl* gene cluster (no induction)
 - E.coli* containing *phl* gene cluster (1mM IPTG)
3. Grow in *E.coli* and *P.putida* in 37 °C and 30°C respectively
4. Grow for 48 hours
5. Sample whole agar plate after 48 hours

DAPG extraction from agar

- Extract with 80% acetone, use enough to cover all the agar
- Evaporate acetone
- Acidify water fraction to 0.1% TFA(v/v)
- Add 1:1 ratio ethyl acetate
- Shake well in 250ml bottles, loosen the cap once in a while to get the gas out
- Place in freezer overnight
- Pour unfrozen fraction to new glassware
- Evaporate ethyl acetate
- Resuspend in 0.5 ml 100% Methanol
- Save sample in fridge for HPLC



BANANA

GUARD

Protocols



DAPG production liquid culture

*note: not all pseudomonas strain produce 2,4-DAPG under liquid culture conditions

- Inoculate cultures in LB +/- antibiotic overnight
- Use overnight culture to inoculate with starting OD of 0.01
-Inoculated 100ml medium in 1L Erlenmeyer flask
- Incubate for 3-4 hours at 37 °C (*E.coli*) or 30°C (*P.putida*) or until OD reach ~0.5
- Induce with IPTG
- Sample 40ml at t=24h and t=48 hours
- Add equal amount (40ml) of ethyl acetate (extraction)
- Shake well bottle
- Put bottle in freezer overnight
- Pour off unfrozen fraction to new glassware
- Evaporate ethyl acetate
- Resuspend in 0.5 ml 100% methanol
- Save samples in fridge for HPLC analysis

HPLC protocol

- We use a Polaris C18-A column (Polaris, Agilent)
- Running from solvent A (25% MeOH 0.03% TFA) to solvent b (100% MeOH, 0.03% TFA)
- Flow= 0.40 ml/min
- One run is as follow:

Time (min)	Solvent A	Solvent B	Flow (ml/min)
0	100		0.4
2	100		0.4
5		100	0.4
14		100	0.4
16	100		0.4
20	100		0.4

1. Retention time of DAPG is around 11.5 min

References:

1. Zhou, Tian-Tian, et al. "phIF mutant of *Pseudomonas fluorescens* J2 improved 2, 4-DAPG biosynthesis and biocontrol efficacy against tomato bacterial wilt." *Biological Control* 78 (2014): 1-8.
2. Schouten, Alexander, et al. "Defense responses of *Fusarium oxysporum* to 2, 4-diacetylphloroglucinol, a broad-spectrum antibiotic produced by *Pseudomonas fluorescens*." *Molecular plant-microbe interactions* 17.11 (2004): 1201-1211.

