Small Scale Agar Extraction

1- Plate out transformants (both empty vector & vector containing the biosynthetic pathway) & DH5α cells.
    a. Incubate LB (LB + antibiotic) plates alongside the inoculated cultures (negative control).
2- After 24 hrs, cut agar plugs (approximately 1 cm by 4 cm) from each of the plates.
3- Mash up agar plugs with glass stirrer & add 5 mL of ethyl acetate.
    a. Incubate at room temperature for 30 mins.
    b. Also have a negative control of no agar.
4- Transfer 2 mL of extract (using a glass pipette) to a 2 mL Eppendorf and spin down the extract at 13,200 rpm for 5 mins.

HPLC-MS Preparation

1 – Transfer 1 mL of crude extract to a fresh Eppendorf & rotavap each sample (40°C for 3 hours).
2 – Re-suspend extract with 200 µL of acetonitryl.
3 – Dilute extracts (1/10) and analyse using HPLC-MS.

HPLC-MS Protocol

"5 µL of extract were eluted at 0.2 mL/min with a solvent composition of 55% methanol & 45% dwater was applied to a HICHROM ACE 3 micron C18 reverse phase column (2.1 mm by 100 mm). A linear gradient of 100% methanol was used for 18 mins. A further 7 mins of 100% methanol gradient was applied to the column. A final solvent composition 55% methanol/45% dwater was applied for 7 mins.

A positive ion electrospray mass spectrometry protocol was used. A nebulizer He gas (flow rate of 20 L/min) was used. A drying flow rate of 6 L/min at 330 °C. A wide automatic tuning range was used in order to detect secondary metabolites" – Craig 2014

Sections need to be revised as 5-HT is small & polar! - Lowden