Resting Cell-Assay [1]:

Materials Needed:
M9 minimal salt media
Chloramphenicol (25mg/ml)
1M IPTG
200 mM 6-CNA dissolved in methanol
250 mL Erlenmeyer flasks with 50 mL LB
2L Erlenmeyer flasks with 1L of LB
1L centrifuge bottles
1L Separatory Funnel
500mL round bottom flask
Ethyl acetate
12M HCL
Anhydrous Magnesium Sulfate
Filter paper
Glass funnel
Rotary evaporator
Pyridine
GC vials, micro-inserts and caps
Nitrogen Gas
BSTFA + TMSC 99:1 (Derivatizing Agent)
Vortex
Spectrophotometer
Cuvette
Centrifuge
pH paper
GC/MS
Pipets

M9 minimal salt media components:
33.9g/L Na_2HPO_4
15g/L KH_2PO_4
5g/L NH_4Cl
2.5g/L NaCl

Protocol:

1) A starter culture is made by picking an isolated colony of DH5-αE.coli transformed with a CCH2 vector from a chloramphenicol plate. The colony was picked with a pipet and rinsed in 50mL of LB with 25ug/mL chloramphenicol in a 250 mL Erlenmeyer flask. The culture was incubated overnight on the shaker at 37°C.
2) Repeat with a no insert colony as a control.
3) Transfer 10 mL of starter culture into 1L of LB with 25ug/mL chloramphenicol and incubate on the shaker at 37°C

4) When the cultures reached an OD600= 0.6-0.8 the cultures are put on ice for 20 minutes and stirred every couple of minutes.
5) After being cooled, 1mL of 1M IPTG was added.
6) The cultures are grown overnight on the shaker at 25°C.
7) Cells are pelleted in 1L centrifuge bottles by centrifuging for 20 minutes on maximum speed at 4°C.
8) The supernatant is discarded from both cultures (CCH2 and No insert) and the cell pellets are washed by resuspending in 500mL of M9 minimal salt media.
9) Cells were pelleted again for 20 minutes on maximum speed at 4°C.
10) The supernatant is discarded again and the cells re-suspended in 200mL of M9 minimal salt media.
11) 250 µL of 200mM 6-CNA was added and the cultures are left overnight at 30°C for 48 hours for the assay.
12) Cells are pelleted in 1L centrifuge bottles for 20 minutes on maximum speed at 4°C.
13) The supernatant was transferred into a Separatory funnel.
14) A liquid-liquid extraction [2] was done with a modified protocol of using larger volumes of ethyl acetate and 12 M hydrochloric acid. The extraction is done twice, keeping the ethyl acetate layer.
15) Anhydrous magnesium sulfate is added to the ethyl acetate layer to dry any water and gravity filtered into a 500mL round bottom flask.
16) The ethyl acetate layer is evaporated via a rotary evaporator.
17) The remaining residue is resuspended in 600 µL of pyridine and transferred into a GC vial to be analyzed on the GC-MS [3].

References:

[1]Cloning of a Novel 6-Chloronicotinic AcidChlorohydrolase from the Newly Isolated 6-Chloronicotinic Acid Mineralizing Bradyrhizobiaceae Strain SG-6C- Madhura Shettigar.

Other Protocols:

[2] Liquid-Liquid Extraction for isolation of 6-Chloronicitinoic Acid and 6-Hydroxynicitinoic Acid protocol.

[3] Gas Chromatography-Mass spectroscopy conditions to run 6-Chloronicitinoic Acid and 6-hydroxynicitinoic Acid and sample preparation: