WEB337 - *in vivo* biotransformation

using SOMT-2

Benjamin Weigel

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1 Introduction

Test whether different substrates available in-lab are converted by SOMT-2 in vivo. Use SOMT seed culture to inoculate main cultures. Add substrates after 4 hours of incubation at 30 °C. 16 substrates means 16 flasks. Take two samples frome each flask at 0, 10, 20 and 30 hours. $16 \times 4 \times 2 = 128$ samples.

2 Experimental

2.1 seed culture

1) ∼10 mL pre-culture in LB supplemented with proper AB (100 ug/mL kanamycin)
2) grow over night at 30 °C and 220 rpm

2.2 main culture

1) pellet cells (5 min @ 5000×g, 4 °C) and wash with 15 mL PBS
2) resuspend pellet in the 3 mL of PBS

!! measure and record OD^{600}

3) inoculate 200 mL of autoinduction medium (+ 100 ug/mL kan) to an $OD^{600} = 0.1$
4) aliquot 10 mL into new flasks for each sample (17 flasks) (*use 100 mL flasks*)
5) add 0.1 mM of flavonoid (see 4) from 10 mM stock in MeOH or DMSO to the cultures at 4 hours after inoculation ($OD^{600} \sim 0.8$)

6) take a 600 μl sample at 10, 20 and 30 hours after inoculation and divide as follows: (**on ice!**)
   a) measure OD^{600} (∼100 μl)
   b) 500 μl for HPLC (see 2.4)

2.3 OD^{600} measurements

- measure OD^{600} in MTP (all samples, 100 μl of sample) **!pathlength differs from cuvette!**
- measure OD^{600} of random samples in cuvette as reference

2.4 HPLC

1. extract 500 μl of culture **twice** with 500 μl ethyl acetate + 1% formic acid
2. vortex for 30 s to extract, centrifgure for 10 min @ 10.000×g, 4 °C to separate phases
3. pool organic phases and evaporate in SpeedVac (45 °C)
4. solve remainder in 200 μl MeOH
5. analyze via HPLC

   Column:
   Injection volume: 10 μl
   Solvent A: H_2O + 0.2% formic acid
   Solvent B: MeCN + 0.2% formic acid
   Program: 5% B (hold 4 min) → 21 min ramp → 100% B (hold 5 min)

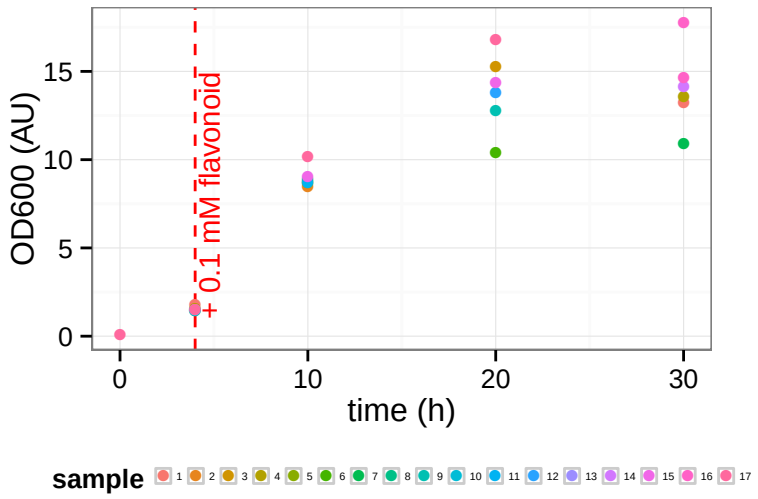
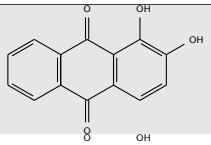
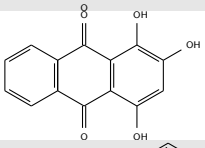
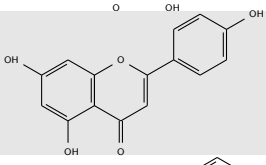
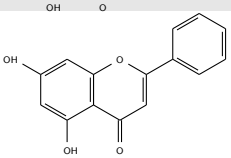


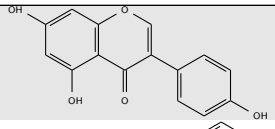
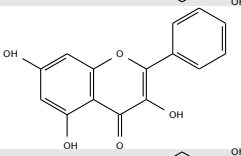
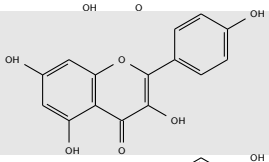
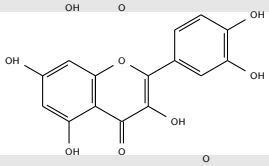
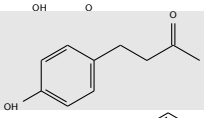
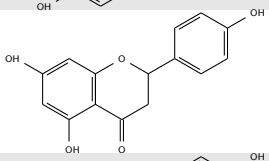
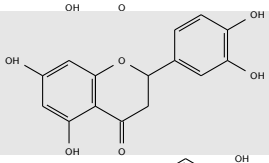
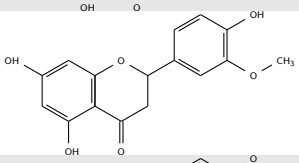
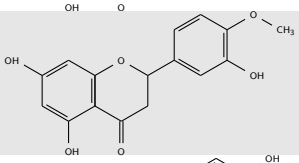
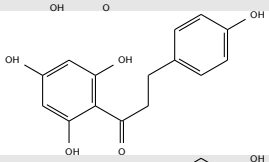
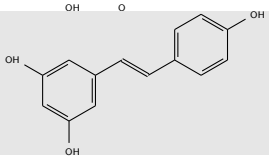
Figure 1: OD^{600} of samples.

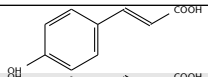
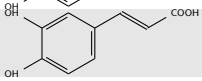
3 Results

3.1 OD^{600}

4 Appendix

No.	substrate	moiety	structure
1	alizarin	anthrachinone	
2	purpurin	anthrachinone	
3	apigenin	flavone	
4	chrysin	flavone	

No.	substrate	moiety	structure
5	genistein	isoflavone	
6	galangin	flavonol	
7	kaempferol	flavonol	
~~8~~	~~quercetin~~	~~flavonol~~	
8	reosmin	homo-dihydro cinnamic keton	
9	naringenin	flavanon	
10	eriodictyol	flavanon	
11	homoeriodictyol	flavanon	
12	hesperetin	flavanon	
13	phloretin	chalcon	
14	resveratrol	stilbene	

No.	substrate	moiety	structure
15	*p*-coumaric acid	cinnamic acid	
16	caffeic acid	cinnamic acid	
17	none (blank)		