Growth inhibition assay

Aim

- To establish whether the transformation products (TPs) generated from the reactions with laccase, SMX with or without the mediator ABTS are more toxic than pure SMX
- To test if the bacteria *Bacillus subtilis (B. subtilis)* and *Escherichia coli (E. coli)* can be used

Materials

- *B. subtilis* strain ISW1214, *E. coli* strain TOP10
- LB media (see separate protocol for making LB media)
- TPs from reactions (see separate protocol for making reactions)
- 96 well plate
- FLUOstar Omega analyser (BMG LABTECH)

Procedure (from plates)

- Streak *B. subtilis* and *E. coli* on separate LB plates (without antibiotics) and incubate the plate overnight
- Pick cells and inoculate in 20 mL of LB media (two separate shaker flasks)
- Let the cells grow overnight at 37 °C, between 150-200 rpm
- Dilute the *B. subtilis* and the *E. coli* to an OD600 of 0.1/ mL LB media
- Pipette 185 µL of cells into wells, and 15 µl of transformation products or pure SMX on the cells
- Pipette them into a 96 well plate and measure OD600 in a plate reader (FLUOstar Omega, BMG LABTECH) every 5 minutes for 7 hours

Notes

Make all samples in triplicates (n=3). Use a LB medium triplicate as a blank. Use pure SMX and cells as control. Pipette pure cells into a triplicate to compare with “ultimate” growth.

After culturing the cells overnight on the plate, it is possible to store the plates in 4 °C for further use.

References

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