Privileged and Confidential: *Baudoinia* germination protocol

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Strain info:

Baudoinia panamericana

Strain: UAMH10762

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Protocol:

Replication of Ewaze, *et al.*, 2008 "Effect of ethanol vapour on dormant cell germination"

Ewaze, J. O., Summerbell, R. C., & Scott, J. A. (2008). Ethanol physiology in the warehouse-staining fungus, Baudoinia compniacensis. Mycological Research, 112(11), 1373–1380. https://doi.org/10.1016/j.mycres.2008.05.003

Design

6 Treatments (Sterile control, Acetone 10ppm control, Ethanol 0.1ppm, Ethanol 1ppm, Ethanol 10ppm, Ethanol 100ppm)

5 replicate subjects per treatment

Grow dormant colonies on sterile filters for 14 days in vapor treatment chambers

Count resultant colonies on filters

Analyze with One-way ANOVA and GLM (Colony count ~ treatment)

Workflow:

1. Order *B. panamericana* strain UAMH10762
2. Prepare solid growth media - ML Agar (see notes)
3. Culture UAMH10762 for bulk growth on ML Agar

4. Calculate and prepare chamber treatments
5. Apply 300 colony-forming units of fungi to each of 30 sterile cellulose filters
6. Allow 14 days of growth in treatment chambers at 26 deg C
7. Remove filters and visualize under light microscopy
8. Analyze data (ANOVA + Tukey Test; Generalized linear regression)
9. Prepare report

Budget ($4,751 - $4,791 total):

Fungal culture ($495 - $535)

- Live culture: $325
- Container: $25
- Shipping: $85 - $125
- Foreign export fee: $15
- Bank transfer fee: $45

Cellulose membrane filters ($56)

- Fisher Scientific MEMBR FLTR 0.45UM 25MM 100/PK

Experimental protocol ($4200)

- Experiment preparation (incl. media prep and culturing): 4 hours
- Experiment setup: 3 hours
- Data collection (colony counting): 4 hours
- Data analysis and reporting: 3 hours

Consulting rate of $300 / hr

Notes:

ML Agar recipe

6.25 g maltose, 6.25 g malt extract 0.63 g, $MgSO4 \cdot 7H2O$, 1.25 g KH2PO4, 0.63 g Bacto peptone, 1 g yeast extract, and 15 g agar in 1000 mL DI Water

Ethanol volumes from Ewaze, *et al.*, 2008

Using the Henry's law constant for ethanol in aqueous solution (5 x 10-6 atm m3 mole-1 at 25 deg C) (Gaffney et al. 1987), ethanol concentrations were calculated to obtain ethanol vapour levels in the head space ranging from 0.1-100 ppm; the actual volumes of 95% ethanol added to the water were 1.23, 12.3, 123 and 1230 uL.

Will need to recalculate for Orem, UT elevation and for Acetone treatment