

Temperature Gradients Liquid Cultures: Planning the Experiment

June-03-16

11:45 AM

Seem to be a good way to test symbiont hypothesis/association with eucaryotes (fungi)

- Do 5g of soil, add water later to cover it up, vortex, autoclave test tubes (17 found)
(we assume all is dead, autoclave water/PBS/Pyrophosphate etc to make dilutions and controls)
- Use 1:1000 dilution 100 ul of the inoculum, vortex everything
- Take samples from some day 0 tubes (representative temperatures, including controls)
- Compare control (no temperature shock) and temperature shock samples as to WPS2 viability, abundance etc
- No additional nutrient source, since previous enrichment showed H₂O as an optimal stimulant
- NM3B soil is used

Proposed Sample table taking into account preliminary results obtained [earlier](#)

- No need to do 55C & 85C & 1' since they are less informative)
- One A sample (3') used just to provide a middle ground for between the non heat shock samples
- Do heat shock and dilutions in non-pyrophosphate PBS!!! (since it could increase viability)

Heat Shock Samples (duplicates are designates as a, b)

2x: 12 Total	2'	3'	4'
A: 55		A3A, A3B	
B:65	B2A, B2B	B3A, B3B	B4A, B4B
C:75	C2A, C2B	C3A, C3B	

Non-heat Shock Samples and Controls

4 samples Total	
Inoculant	0'
1:1000, 100uL	S0A, S0B
H ₂ O, 100uL	W0A, W0B

Overall: 16 samples

Detachment Procedure: Added Pyrophosphate

From Eichorst et al 2007 AEM <http://aem.asm.org/content/73/8/2708.full>

Approx 30g of soil was added to 100 ml of a phosphate-buffered salts solutions

- 137 mM NaCl
- 2.7 mM KCl
- 10 mM Na₂HPO₄
- 2 mM KH₂PO₄

pH was adjusted to 7.0, and the solution was supplemented with 2.24 mM Na₄P₂O₇·10H₂O as a dispersal agent

(~~dithiothreitol 1 mM as a reducing agent~~): we are not doing that

The soil suspension was stirred vigorously with a magnetic stir bar for 30 min. Senser soil aggregates were then allowed to settle for 30 min, after which an aliquot of the supernatant fraction was used for total direct cell counts after staining with 5-(4,6-dichlorotriazine-2-yl) aminofluorescein

For 50 ml:

137 mM NaCl: mw 58.44 = 0.4 g

2.7 mM KCl, at mw 74.55 = 0.010 g

10 mM KH₂PO₄ mw 136 = 0.068 g

2.24 mM Na₄P₂O₇·10H₂O mw 446 = 0.05 g