Bold's Nutrient Media with Nitsch's Micronutrients
For culturing autotrophic fern gametophytes

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STOCK SOLUTION
10g $NaNO_3$ in 400 ml RO H_2O
1g $CaCl_2$ in 400 ml RO H_2O
3g K_2HP0_4 in 400 ml RO H_2O
7g KH_2PO_4 in 400 ml RO H_2O
3g $MgSO_4*7H_20$ in 400 ml RO H_2O
1g NaCl in 400 ml RO H_2O

Store prepped stock solutions in media storage bottles.

NITSCH'S MICRONUTRIENTS
To 1L of RO H_2O, add the following:

0.5 mL H_2SO_4 (conc.)
3 g $MnSO_4$ * 4 H_2O **or** 2.27g $MnSO_4$ * 1 H_2O
0.5 g $ZnSO_4$ * 7 H_2O
0.5 g H_3BO_4
25 mg $CuSO_4$ * $5H_2O$
25 mg $CoCl_2$
25 mg Na_2MoO_4 * $2H_2O$

Store prepped stock solution in media storage bottle.

ADDITIONAL COMPONENTS
0.7% agar
1% $FeCl_3$ solution

MEDIA PREPARATION
For 1L of solution:

1. Add 10mL of each stock solution to 1L volumetric flask.

2. Add 2mL of micronutrient solution to flask.

3. Add 1 drop of 1% $FeCl_3$ solution to flask (Do not add if non-culture grade reagents are used).

4. Add 7g agar to flask.

5. Bring flask to volume with RO H_2O. Add H_2O gradually and swirl flask to suspend agar in solution.

6. Transfer solution to 1L media storage bottle, swirling flask occasionally to resuspend agar. Cap bottle loosely (see next step).

7. Autoclave bottle and solution for ~30 min. If cap is on tight, the bottle will break in autoclave and spill solution. Keep bottle in autoclave for ~45min following cycle to allow bottle to cool to 50-60°C.

8. While media is in autoclave, lay out 25-30 (number needed varies based on amount used) 100 x 25mm Petri dishes along edge of lab desk. Place lids next to plates.

9. Remove media from autoclave and pour into Petri dishes. Fill each dish ~2/3 of the way. Replace lids on dishes – they should steam up. This will sterilize them. Allow dishes to cool on lab bench overnight. Once media has cooled and solidified, remove lids individually, remove any water that has condensed on the lid, and return lid. Excess water will interfere with spore sowing and germination. If dishes are not used immediately, place in crisper box and refrigerate.