McKay Agar Recipe

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Background

- The following media selects for growth of organisms in the Streptococcus Milleri Group, ie. *Streptococcus anginosus, Streptococcus constellatus* and *Streptococcus intermedius*.

Equipment

- Scale, stir plate
- Autoclave

McKay Agar Protocol:

Used to prepare 3L of media

1. Prepare the following:

   Hemin
   - add 0.015g to 1mL of 1M NaOH
   - bring up to 30mL with ddH_2O (final NaOH conc is 0.1M)
   - use fresh

   100x Colistin
   - dissolve 0.03g of colistin sulfate (Sigma#C4461) in 30mL ddH_2O
   - wear gloves
   - filter sterilize
   - use fresh

   100x Oxolinic Acid
   - dissolve 0.015g in 30mL of 0.1M NaOH
   - wear gloves
   - filter sterilize
   - use fresh

   L-Argenine-HCl (2.5%)
   - add 1.5 g of L-Arg-HCl to 60mL ddH_2O
   - filter sterilize
   - can be made ahead of time and in a larger volume (store at RT short term or 4°C long term)

   Sulfadiazine (16mg/mL)
   - 1.6g added to 90mL of 0.1M NaOH
   - once dissolved top up to 100mL

- filter sterilize
- can be made ahead of time and in a larger volume (store at RT short term or 4°C long term)

Salt Solution
- can be made ahead of time and stored at RT
- always check before use. If cloudy or crystals form, make fresh.
- Add:
5g $NaHCO_3$
1g NaCl
0.5g K_2HPO_4
0.5g KH_2PO_4
0.25g $MgSO_4.7H_2O$
500mL H_2O
- filter sterilize

1M $CaCl_2$
- make a 20-30mL stock
- filter sterilize

2. Prepare Solution A
   - 40g of nutrient broth (Difco) in 1L of dH_2O
   - mix well

3. Using a widemouth 2.8L flask mix the following:
   - 15g Glucose
   - 30g Yeast extract
   - 15g Tryptone
   - 6g K_2HPO_4
   Dissolve in 600mL dH_2O with a stir bar

4. Add while stirring:
   - 3mL Tween-80 using a 18G syringe and needle (tween is thick and will not go in a micropipette tip)
   - 120mL salt solution
   - Solution A
   - 300µl of 1M $CaCl_2$ (add slowly to prevent any precipitation of the salts)
   - Add 30mL of Hemin
   - 3µL of conc. Vitamin K (Sigma V3501). Ensure pipette goes directly into broth and pipette up and down to get all of it out. Use gloves and be careful with the volume since Vitamin K is thick and will suck up slowly in the pipette.

5. Bring volume up to 2800mL and transfer into a large 6L flask
   - pour into 1L graduated cylinder to transfer
   - add water into 2.8L flask to rinse it out and then use grad. cylinder to transfer the remaining

1.8L

6. Add 45g of Bacto Agar (Difco)

7. Check the pH the first time you make this to ensure it is around 7.2 (once you are sure your pH is correct it should not be necessary to re-pH each time the agar is made)

8. Add indicator dyes:
   - 0.18g bromocresol purple
   - 0.003g of crystal violet
   accuracy is very important here, be as close as possible to these weights and ensure all powder is removed from weigh sheet

9. Autoclave and allow media to equilibrate to 55°C in a water bath

10. While stirring add:
    -60mL of L-Argenine-HCl
    -30mL of 100x Colistin
    -30mL of 100x Oxolinic acid
    -93.75mL of Sulfadiazine (if you made a 100mL then you would essentially use the entire amount since some liquid is lost during the sterilization)

11. Allow additives to mix in for ~5min

12. Pour 30mL per plate

13. In order to ensure the media was prepared properly it is advised to QC using the 3 SMG sequenced strains (B196 *S. intermedius*, C238 *S. anginosus*, C232 *S. constellatus*)

REFERENCES

1. Sibley, C.D., Parkins, M.D., Duan, K. Norgaard, C.J, Rabin, H. and Surette, M.G. (2008). A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. ***Proc. Natl. Acad. Sci. USA*** 105:15070-15075

2. Sibley C.D., Grinwis, M.E., Field, T.R., Parkins M.D., Norgaard, J.C., Gregson, D.B., Rabin H.R. and Surette M.G. McKay Agar Enables Routine Quantification of the *Streptococcus milleri* Group in Cystic Fibrosis Patients. (2010) ***J. Med. Micro.*** 59:534-40