Microbial broths and agar recipes

Unless otherwise stated all solutions are sterilised by autoclave to 20 min at 121°C.

5% PTYG medium and agar

Add to 1L DI water: 0.25 g peptone, 0.25 g tryptone, 0.5 g yeast extract, 0.5 g glucose, 0.6 g $MgSO_4 \cdot 7H_2O$, 0.07 g $CaCl_2 \cdot 2H_2O$ and 15 g agar (for agar)

Also modified by spreading 0.5mL of 1M HCl or NaOH after plates set (20 mL volume).

Actinomycete isolation agar (Sigma Aldrich) prepared according to manufacturer's instructions

Also modified by spreading 0.5mL of 1M HCl on the surface of each plate.

CAB

Add to 1L DI water: 140 g glucose, 2.5 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.5 mg $FeSO_4 \cdot 7H_2O$ and 1.3 mg $ZnSO_4 \cdot 7H_2O$

Horikoshi agar

Add to 950 mL DI water: 5 g peptone, 5 g yeast extract, 10 g glucose, 0.2 g $MgSO_4 \cdot 7H_2O$, K_2HPO_4 and 20 g agar

Dissolve in 50 mL DI water: 5 g $NaCO_3$

Autoclave separately and mix aseptically before pouring plates

Modified CAB

Add to 1L DI water: 140 g glucose, 2.5 g $(NH_4)_2SO_4$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.5 mg $FeSO_4 \cdot 7H_2O$ and 1.3 mg $ZnSO_4 \cdot 7H_2O$

PTYG broth

Add to 1L DI water: 5 g peptone, 5 g tryptone, 10 g yeast extract, 10 g glucose, 0.6 g $MgSO_4 \cdot 7H_2O$ and 0.07 g $CaCl_2 \cdot 2H_2O$

Rose Bengal agar

Add to 1L DI water: 5 g peptone, 10 g glucose, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g KH_2PO_4, 0.05g Rose Bengal and 15 g agar

Urease agar

Add to 950mL DI water: 1 g peptone, 0.8 g KH_2PO_4, 1.2 g Na_2HPO_4, 0.012 g phenol red and 15g agar

Dissolve in 50mL DI water: 20 g urea, filter sterilised

Allow first solution to cool to about 50°C before mixing with urea

Characterisation of microbial properties

pH drop

5% PTYG plates with 0.04 % bromocresol purple

Looking for a colour change around colonies from blue/purple to yellow, with colour change between pH 6.8 to 5.2.

Urease activity

Make urease agar as above. Urease activity indicated by colour change in agar from yellow to red

Inorganic phosphate utilisation (NBRIA)

Add to 1L DI water: 10 g glucose, 0.25 g $MgSO_4 \cdot 7H_2O$, 5 g MgCl, 0.2g KCl, 0.1 g $(HN_4)_2SO_4$, 5 g Ca_3PO_4, 0.025 g bromophenol blue and 15g agar

Note: Ca_3PO_4 will not dissolve. Mix well to distribute evenly before pouring plates.

Inorganic phosphate utilisation is indicated by clearing of insoluble phosphate around the colony and colour change from blue to yellow

Siderophore activity

21.9 mg HDTMA in 50 mL DI water (solution 1)
1.5 mL 1 mM $FeCl_3 \cdot 6H_2O$ in 10 mM HCl (solution 2)
7.5 mL 2 mM CAS (solution 3)
4.307g PIPES in 30 mL DI water (solution 4)
0.2M sulfosalicylic acid (solution 5)
Mix solutions 2 and 3, and slowly add to solution 1, add solution 4 then make up to 100 mL with DI water
Microbial isolates were grown in 5% PTYG for 7 days, then spun at 16000 x g for 5 mins
Mix 0.5mL solution with 0.5mL supernatant add 10µl solution 5
Siderophore activity is indicated by a colour change from blue, usually to pink or orange

PCR and sequencing conditions

PCR was carried out using 16s rRNA gene primers 27f (AGAGTTTGATCMTGGCTCAG) and 1387r (GGGCGGWGTGTACAAGGC) using the following PCR program: 3 min 95°C, followed by 35 cycles of 95°C for 30 s, 66°C for 30 s, and 72°C for 120 s, with a final extension period of 5 min at 72°C. Sequencing was conducted by Eurofins Genomics on PCR products using primers 515f (GTGCCAGCMGCCGCGGTAA) and 907R (CCGTCAATTCMTTTRAGTTT). DNA from fungal isolates were amplified using EUK1AF (CTGGTTGATCCTGCCAG) and EUK516R (CCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGACCAGACTTGCCCTCC) primers for the 18s rRNA using the following program: 3 min 95°C, followed by 40 cycles of 95°C for 30 s, 56°C for 50 s, and 72°C for 30 s, with a final extension period of 5 min at 72°C. The PCR

product was sequenced using EUK1AF and EUK516F primers. Fungal identification was also conducted using primers covering the internally transcribed sequence between the 18s and 5.8s subunits (ITS1) using primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) using the following program: 5 min 95°C, followed by 30 cycles of 95°C for 30 s, 60°C for 30s, and 72°C for 30 s, with a final extension period of 5 min at 72°C. The PCR product was sequenced using ITS1 and ITS2 (GCTGCGTTCTTCATCGATGC) primers.