3: AZOTOBACTER MEDIUM

Glucose	5.00	g
Mannitol	5.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
Na_2MoO_4 x 2 H_2O	5.00	mg
K_2HPO_4	0.90	g
KH_2PO_4	0.10	g
$FeSO_4$ x 7 H_2O	0.01	g
$CaCO_3$	5.00	g
Agar	15.00	g
Distilled water	950.00	ml

1. Adjust pH to 7.3.

2. Sterilize glucose and mannitol separately (in 50 ml H_2O) and add to the medium after autoclaving. Calcium carbonate in the medium serves as a buffer. The calcium carbonate will settle in agar plates before the agar has set, producing an opaque layer in the bottom. As the strain grows and acid is produced this will react with the calcium carbonate, causing it to dissolve and form zones of clearing immediately below the colonies.