**ATCC medium: 1277 *Propionispira* medium (ATCC medium 1045) with 0.1% yeast extract and 0.4% sodium lactate**

ATCC Medium 1045 (see below) with 0.1% yeast extract and 0.4% sodium lactate

*ATCC Medium 1045:*
*Mineral Solution 1:*
$K_2HPO_4$ .....6.0 g
Distilled water.....1.0 L

*Mineral Solution 2:*
$KH_2PO_4$ .....6.0 g
$(NH_4)_2SO_4$.....6.0 g
NaCl.....12.0 g
$MgSO_4 . 7H_2O$ .....2.4 g
$CaCl_2 . 2H_2O$ .....1.6 g
Distilled water.....1.0 L

*Sodium Carbonate Solution:*
$Na_2CO_3$ .....8.0 g
Distilled water.....100.0 ml

*Reducing Agent:*
Add 300 mg L-Cysteine . HCl to 10 ml of water and 300 mg $Na_2S . 9H_2O$ to a second 10 ml of water: tube under nitrogen gas. After autoclaving at 121C for 15 minutes under fast exhaust and allowing to cool, mix equal amounts of the cysteine and the sodium sulfide (under nitrogen). The mixture should be clear and can be used up to two weeks.

Prepare the complete medium by mixing the above components in the following proportions:
83.5 parts of distilled water
5.0 parts of Mineral Solution 2
2.5 parts of Mineral Solution 1
5.0 parts of 8.0% $Na_2CO_3$ Solution
Agar (if needed).....20.0 g
1.0 part of Wolfe's Mineral Solution (see below)
1.0 part of Wolfe's Vitamin Solution (see below)
2.0 parts of Cysteine-Sulfide Reducing Agent
Resazurin (0.025% solution).....4.0 ml/L

Mix all the ingredients except the Wolfe's Vitamin and Cysteine/$Na_2S$ Solutions. After autoclaving for 15 minutes, cool under 80% $N_2$, 20% $CO_2$ and add the Wolfe's Vitamin Solution, then Cysteine/$Na_2S$ Solution. Adjust pH to 7.2 if necessary and tube anaerobically, aseptically.

*Wolfe's Mineral Solution:*
Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....1.5 g
$MgSO_4 . 7H_2O$ .....3.0 g
$MnSO_4 . H_2O$ .....0.5 g
NaCl.....1.0 g
$FeSO_4 . 7H_2O$ .....0.1 g
$CoCl_2 . 6H_2O$ .....0.1 g
$CaCl_2$.....0.1 g
$ZnSO_4 . 7H_2O$ .....0.1 g
$CuSO_4 . 5H_2O$ .....0.01 g
$AlK(SO_4)_2 . 12H_2O$.....0.01 g
$H_3BO_3$ .....0.01 g
$Na_2MoO_4 . 2H_2O$.....0.01 g
Distilled water.....1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

*Wolfe's Vitamin Solution:*
Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Biotin.....2.0 mg
Folic acid.....2.0 mg
Pyridoxine hydrochloride....10.0 mg
Thiamine . HCl.....5.0 mg
Riboflavin.....5.0 mg
Nicotinic acid.....5.0 mg
Calcium D-(+)-pantothenate...5.0 mg
Vitamin B12.....0.1 mg
p-Aminobenzoic acid.....5.0 mg
Thioctic acid.....5.0 mg
Distilled water.....1.0 L