921. METHYLOSARCINA QUISQUILLARUM AND M. FIBRATA MEDIUM

**Solution 1** (10x NMS salts):
- **KNO₃**: 10.00 g
- **MgSO₄ x 6 H₂O**: 10.00 g
- **CaCl₂ x 2 H₂O**: 2.00 g
- **Distilled water**: 1000.00 ml

Dissolve the ingredients listed above (in that order) in about 700 ml of distilled water, and then make up to 1 litre.

**Solution 2** (Fe EDTA):
- **Fe EDTA (Sigma, 03650)**: 3.80 g
- **Distilled water**: 1000.00 ml

**Solution 3** (Sodium molybdate):
- **Na₂MoO₄ x 2 H₂O**: 0.26 g
- **Distilled water**: 1000.00 ml

**Trace elements**:
- **EDTA di sodium salt**: 25.00 mg
- **CuSO₄ x 5 H₂O**: 100.00 mg
- **FeSO₄ x 7 H₂O**: 50.00 mg
- **ZnSO₄ x 7 H₂O**: 40.00 mg
- **H₃BO₃**: 1.50 mg
- **CoCl₂ x 6 H₂O**: 5.00 mg
- **MnCl₂ x 4 H₂O**: 2.00 mg
- **NiCl₂ x 6 H₂O**: 1.00 mg
- **Distilled water**: 100.00 ml

May be stored at 4°C in the dark.

**Phosphate buffer**:
- **Na₂HPO₄ x 12 H₂O**: 71.60 g
- **KH₂PO₄**: 26.00 g
- **Distilled water**: 1000.00 ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Prepare the growth medium as follows:
Dilute 100 ml solution 1 to 1 litre with distilled water and then add 1 ml of solution 3, 1 ml of the trace elements, and 0.1 ml of solution 2. If solid media is required add 1.5% agar. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 50% methane to the gas phase and autoclave at 115°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. When the growth medium is cool 10
ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.

**DSM 111909** may be grown on 1.0% methanol and for solid media use 15g agar per liter.