

## 1464. CHITINIVIBRIO MEDIUM

### Mineral Base:

|                                 |          |
|---------------------------------|----------|
| NaHCO <sub>3</sub>              | 15.0 g/l |
| Na <sub>2</sub> CO <sub>3</sub> | 95.0 g/l |
| NaCl                            | 6.0 g/l  |
| K <sub>2</sub> HPO <sub>4</sub> | 1.0 g/l  |

Adjust pH to 10.0 with NaOH or HCl. Make anoxic under nitrogen stream and autoclave at 121°C.

### After autoclaving add sterile, anoxic solutions of:

|   |                             |
|---|-----------------------------|
| Chitin (amorphous)                            | 1 – 2g/l (from 5% solution) |
| MgSO <sub>4</sub>                             | 1 mM (final concentration)  |
| Yeast extract                                 | 20 mg/l                     |
| NH <sub>4</sub> Cl                            | 4 mM (final concentration)  |
| Na <sub>2</sub> S                             | 1 mM (final concentration)  |
| Acidic trace metals (Pfennig & Lippert, 1965) | 1 ml/l                      |
| Vitamine mixture (Pfennig & Lippert, 1965)    | 1 ml/l                      |

### Acidic trace metals (Pfennig & Lippert, 1965)

|   |           |
|---|-----------|
| 1. EDTA   | 5000 mg/l |
| 2. FeSO <sub>4</sub> x 7H <sub>2</sub> O  | 2000 mg/l |
| 3. ZnSO <sub>4</sub> x 7H <sub>2</sub> O  | 100 mg/l  |
| 4. MnCl <sub>2</sub>  | 30 mg/l   |
| 5. H <sub>3</sub> BO <sub>3</sub>   | 300 mg/l  |
| 6. CoCl <sub>2</sub> x 6H <sub>2</sub> O  | 200 mg/l  |
| 7. CuCl <sub>2</sub>  | 10 mg/l   |
| 8. NiCl <sub>2</sub> x 2H <sub>2</sub> O  | 20 mg/l   |
| 9. Na <sub>2</sub> MoO <sub>4</sub> x 2H <sub>2</sub> O or NH <sub>4</sub> MoO <sub>4</sub> | 20 mg/l   |

adjust pH to 3-4 with HCl; autoclave in closed bottles, 120°C, 20 min.

### Vitamine mixture (Pfennig & Lippert, 1965)

1: acidic solution (HCl, pH 3; 100 ml)

|               |        |
|---------------|--------|
| Thiamin       | 5 mg   |
| Ca pantotenat | 5 mg   |
| B12           | 0.5 mg |

2: basic solution (100 ml 0.1 N NaOH)

|                |       |
|----------------|-------|
| Biotin         | 2 mg  |
| PABA           | 5 mg  |
| Nicotinic acid | 5 mg  |
| Pyridoxine     | 10 mg |

3: Neutral solution (100 ml Aqua dem.)

|            |      |
|------------|------|
| Folic acid | 2 mg |
| Riboflavin | 5 mg |

Sterilize by filtration and keep at 4°C. Before addition to the medium mix 1:1:1 (vol:vol:vol).