

## 1569. ORENIA CHITINITROPHA

**Mineral Base:**

NaCl	120.0	g
K <sub>2</sub> HPO <sub>4</sub>	2.5	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.5	g

Adjust pH to 7.0 with KH<sub>2</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub>. 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)
MgCl <sub>2</sub> x 6H <sub>2</sub> O	5 mM (final concentration)
Yeast extract	20 mg/l
NaHCO <sub>3</sub>	20 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 pantotenate	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).