1712. AROMATOLEUM MEDIUM

Based on the medium in *Rabus and Widdel, 1995, Arch. Microbiol., 163, 96-103*.

1. Anoxic medium

KH_2PO_4	0.5	g
NH_4CL	0.3	g
$MgSO_4$ x $7H_2O$	0.5	g
$CaCl_2$ x $2H_2O$	0.1	g
$NaNO_3$	0.6	g
distilled water	950	mL

The medium was autoclaved and removed from the autoclave at 85°C. Oxygen dissolution was prevented by exchanging the gas-phase with N_2. The medium was complemented by addition of the following stock solutions:

Trace element solution	1.0	mL/L
Vitamin mixture	1.0	mL/L
Vitamin B_{12} solution	1.0	mL/L
1 M $NaHCO_3$	40.0	mL/L

After adding bicarbonate the gas was changed from N_2 to N_2/CO_2 (90/10 %, v/v) and the pH was adjusted to 7.2 – 7.4 with sterile 2 M HCL.

1 M Ascorbate	4.0	mL/L
1 M Benzoate	4.0	mL/L

2. Preparation of stock solutions

Trace element solution

Na_2-EDTA	5.2	g
$FeSO_4$ x $7H_2O$	2.1	g
H_3BO_3	0.03	g
$MnCl_2$ x $4H_2O$	0.1	g
$CoCl_2$ x $6H_2O$	0.19	g
$NiCl_2$ x $6H_2O$	0.024	g
$CuSO_4$ x $5H_2O$	0.029	g
$ZnSO_4$ x $7H_2O$	0.144	g
Na_2MoO_4 x $2H_2O$	0.036	g

The trace element solution was prepared by dissolving the salts in 800 mL warm H_2O_{bidest}
NaOH was used to adjust the pH to 6.5. The solution was added up to 1000 mL with H_2O_{bidest} and autoclaved.

Vitamin mixture

Na_2HPO_4/NaH_2PO_4, 25 mM, pH 7.1	100	mL
4-Aminobenzoic acid	4.0	mg
D-(+)-Biotin	1.0	mg
Nicotinic acid	10.0	mg
Calcium D-(+)-pantothenate	5.0	mg
Pyridoxolhydrochloride	15.0	mg
DL-α-lipoic acid	1.5	mg
Folic acid	4.0	mg
2- Mercaptoethanesulfonic acid, sodium salt	25.0	mg

The solution was filter-sterilized and stored at 4°C in the dark.

Vitamin B_{12} solution

Cyanocobalamine	2.5	mg
H_2O_{bidest}	ad 50.0	mL

The solution was filter-sterilized and stored at 4°C in the dark.

Bicarbonate solution (1M)

$NaHCO_3$	84.0	g
H_2O_{bidest}	ad 1000.0	mL

The solution was dispensed in the required portions (40 mL per 1 L medium). The head space of the bottles was flushed with CO_2. Saturation of CO_2 in the solution was achieved by repeated flushing and shaking of the bottles. Finally, the bottles were sealed with rubber stoppers and aluminum crimps and autoclaved.

Ascorbate solution (1M)

Ascorbic acid	17.6	g
H_2O_{bidest}	ad 100.0	mL

In an ice bath ascorbic acid was dissolved in 80 mL anoxic $H_2O_{bidest\ (autoclaved,}$ cooled under N_2 atmosphere) while flushing with N_2. The pH was adjusted to 7.0 with NaOH and the solution added up to 100 ml with anoxic $H_2O_{bidest.}$ After filter-sterilization the solution was stored under N_2 atmosphere in a special flask (which can be flushed with gas) at 4°C in the dark.

Benzoate solution (1M)

Sodiumbenzoate	14.4	g
H_2O_{bidest}	ad 100.0	mL

The solution was filter-sterilized and stored at room temperature.