1598. VERRUCOMICROBIUM CANDIDATUS MEDIUM (KAM1)

10x NMS salts		
NH_4Cl	10.00	g
$MgSO_4 \times 7\ H_2O$	10.00	g
$CaCl_2 \times 2\ H_2O$	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

FeNaEDTA	4.50	g
Distilled water	1000.00	ml

Trace Element Solution: (see media 1376)		
Na2 EDTA	0.25	g
ZnSO4 x 7 H2O	0.40	g
MnCl2 x 4 H2O	0.02	g
H3BO3	0.02	g
Na2MoO4 x 2 H2O	0.04	g
NiCl2 x 2 H2O	0.01	g
CuSO4 x 5 H2O	0.20	g
CoCl2 x 6 H2O	0.05	g
Distilled water	1000.00	ml

May be stored at 4°C in the dark

Phosphate buffer:		
KH_2PO_4	37.325	g
$Na_2HPO_4 \times 12\ H_2O$	48.950	g
Distilled water	1000.00	ml

Cerium(III)chloride: (0.03M)		
$CeCl_3 \times 7$ H2O	11.2	g
Distilled water	1000.00	ml

Prepare the growth medium as follows:

1. Dilute 100 ml NMS salt solution to 1 litre with distilled water and then add 1 ml of *FeNaEDTA* solution and 1 ml of the trace elements and autoclave. Also autoclave the phosphate buffer stock solution.
2. After cooling of the autoclaved solutions, add 20ml phosphate buffer stock solution to the mineral solution.
3. Dispense 9ml distilled water into the growth vessels. Use sealed vessels it is appropriate to add 10% methane and 1% CO_2 gas to the gas phase and autoclave.
4. When the growth vessels is cool, 1.0 ml of the mineral medium and 0.1 ml 0.03 M Cerium-(III)-chlorid are added.
5. Adjust pH to 3.5 with 1M HCL to obtain the final mineral medium before inoculate.
6. The cultures should be grown with shaking.