

Selective isolation Media

1. Amycolatopsis selective isolation medium (Tan et al., 2002)

1.1 SM1: Steven's medium agar (1x) supplemented with D(+) sorbitol (1% w/v) and neomycin sulfate (4 µg/ml).

1.2 SM2: Steven's medium agar (1x) supplemented with D(+) melezitose (1% w/v) and neomycin sulfate (4 µg/ml).

1.3 Gauze's medium No.2 supplemented with nalidixic acid (10 µg/ml) and novobiocin sodium salt (10 µg/ml).

2. Arginine-Vitamin (AV) agar (Nonomura and Ohara,1969a)

L-Arginine	0.3 g
Glucose	1.0 g
Glycerol	1.0 g
K ₂ HPO ₄	0.3 g
MgSO ₄ .7H ₂ O	0.2 g
NaCl	0.3 g
Agar	15.0 g
Distilled water	1000.0 ml

Trace salt solution

CuSO ₄ .H ₂ O	1.0 mg/ml
Fe ₂ (SO ₄) ₃	10.0 mg/ml
MnSO ₄ .7H ₂ O	1.0 mg/ml
ZnSO ₄ .7H ₂ O	1.0 mg/ml

Vitamin (final weight in medium): 0.5 mg each of p-aminobenzoic acid, calcium pantothenate, inositol, niacin, pyridoxine HCl, riboflavin, thiamine HCl, and 0.25 mg biotin.

Antibiotic:	Cyclohexamide	50 mg
	Nystatin	50 mg
	Nalidixic acid (none or)	20 mg
	Penicillin G (none or)	0.8 mg
	Polymixin (none or)	4.0 mg

pH to 6.4

3. Chitin-V agar (Hayakawa and Nonomura, 1984)

Colloidal chitin	2 g (dry weight)
CaCO ₃	0.02 g
FeSO ₄ .7H ₂ O	10.0 mg
K ₂ HPO ₄	0.35 g
KH ₂ PO ₄	0.15 g
MgSO ₄ .7H ₂ O	0.2 g
MnCl ₂	1.0 mg
NaCl	0.3 g
ZnSO ₄ .7H ₂ O	1.0 mg
Agar	18.0 g
Distilled water	1000 ml

B Vitamins, as for AV agar; cycloheximide 50 mg

pH 7.2

4. Humic acid-vitamin agar

Humic acid	10 g*
Na ₂ HPO ₄	0.5 g
KCl	1.71 g
MgSO ₄ .7H ₂ O	0.05 g
FeSO ₄ .7H ₂ O	0.01 g
CaCO ₃	0.02 g
B-vitamins**	
Cycloheximide	50.0 mg
Agar	18.0 g
Distilled water	1000.0 ml

pH 7.2

* Dissolved in 10 ml of 0.2 N NaOH

** 0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxine-HCl, inositol, Ca-pantothenate, p-aminobenzoic acid and 0.25 mg of biotin.

B-vitamins and cycloheximide were filter-sterized.

5. Inorganic salt-starch agar (Kuster, 1959)

Solution I:	Difco soluble starch	10.0 g
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Make a paste of the starch with a small amount of cold distilled water and bring to volume of 500 ml.

Solution II:	CaCO ₃	2.0 g
	K ₂ HPO ₄ (anhydrous salts)	1.0 g
	MgSO ₄ .7H ₂ O	1.0 g
	NaCl	1.0 g
	(NH ₄) ₂ SO ₄	2.0 g
	Distilled water	500.0 ml
	Trace salt solution*	1.0 ml

Adjust to pH 7-7.4 Mix solutions I and II and add 20.0 g agar.

*Trace salt solution:	FeSO ₄ .7H ₂ O	0.1 g
	MnCl ₂ .4H ₂ O	0.1 g
	ZnSO ₄ .7H ₂ O	0.1 g
	Distilled water	100.0 ml

6. Medium No1 (Gause et al, 1957)

Starch	20.0 g
FeSO ₄ .7H ₂ O	0.01 g
KNO ₃	1.0 g
K ₂ HPO ₄	0.5 g
MgSO ₄	0.5 g
NaCl	0.5 g
Agar	30.0 g

pH 7.2-7.4

7. Medium No.2 (Gause et al, 1957)

Glucose	10.0 g
Peptone	5.0 g
NaCl	10.0 g
Tryptone	3.0 g
Agar	15.0 g

8. MGA-SE Agar (Nonomura and Chara, 1971b)

L-asparagine	1.0 g
Glucose	2.0 g
K ₂ HPO ₄	0.5 g
Soil extract	200.0 ml
Distilled water	800.0 ml
Agar	20.0 g
Antibiotic:	
Cycloheximide	50 mg
Nystatin	50 mg
Benzylpenicillin	0.8 mg
Polymixin B	4.0 mg

Soil extract: 1000.0 g soil in 1L water, autoclaved for 30 minutes, the decanted and filtered.
Final pH 8.0.

9. SM 1

Basal medium	
Yeast nitrogen base	67 g
Casamino acid	0.1 g
Distilled water	1000.0 ml
Sterilized by filter with cellulose filter	
Steriled dipotassium hydrogen phosphate	200.0 ml (10%w/v)

One hundred basal medium was added to 900 ml sterile molten agar (1.5%w/v) followed by filter sterilized solution of D(-) sorbitol (final concentration) 1% w/v, cycloheximide 50µg/ml, neomycin sulphate 4µg/ml and nystatin 50µg/ml.

9. Starch casein + novobiocin agar (Kuster & Williams 1964)

Difco vitamin-free casein	0.3 g
KNO ₃	2.0 g
NaCl	2.0 g
MgSO ₄ .7H ₂ O	0.05 g
CaCO ₃	0.02 g
FeSO ₄ .7H ₂ O	0.01 g
Soluble starch	10.0 g
Agar	15.0 g
Distilled water	1000.0 ml
pH 7.0-7.2	

All the salts were dissolved in 1000 ml of water and pH adjust to 7.0-7.2. Agar was added and the solution steamed to dissolve at 121oC for 20 minutes. After autoclaving, the following was added before pouring into Petri dishes: cycloheximide 25 µg/ml, nystatin µg/ml and novobiocin-sodium salt 25 µg/ml.

10. Stevenson's medium (Stevenson, 1967)

Yeast nitrogen base	67.0 g
Casamino acids	0.1 g

Dissolved them to 1000 ml distilled water and filter sterilized

Sterilized dipotassium hydrogen phosphate (200 ml, 10% w/v) was added to 800 ml of the preparation to make a 10x solution of Stevenson's medium.

For a 1x Stevenson's medium: 100 ml of 10x Stevenson's medium was added aseptically to 900 ml sterile molten agar (1.5% w/v).

11. Streptomyces isolation medium + oxytetracyclin (Kim et al., 2010)

Casein	0.4 g
Starch	1.0 g
CaCO ₃	0.1 g

KH ₂ PO ₄	0.2 g
KNO ₃	0.5 g
MgSO ₄	0.1 g
Agar	15.0 g

All the salts were dissolved in 1000 ml of distilled water. Agar was added and the solution steamed to dissolve the agar. The medium was dispensed into 1000 ml bottle and autoclaved at 121°C for 20 minutes. After autoclaving, the following were added to the bottle before pouring into Petri dishes: oxytetracycline 20 µg/ml and cycloheximide 25 µg/ml.

12. Oligotrophic medium (www.falw.vu/~microb/Protocols/Media_and.../oligotrophic-medium.pdf)

NaHCO ₃	3.8 g
NH ₄ Cl	2.0 g
100 x TY	10 ml
100 x Cl-salts	10 ml
100 x P	10 ml
Agar	15.0 g
dH ₂ O	970 ml

solutions:

100 x TY: 2.5% yeast extract, 2.5% tryptone

100 x Cl-salts: 1.0% MgCl₂, 0.5% CaCl₂

100 x P: 0.4 % KH₂PO₄