Aim 47 To Prepare MS Medium

Introduction

Now a day dry powdered medium containing inorganic salts, vitamin and amino acids are available commercial in the market. So the culture media are prepared by dissolved the power in D.W. and after adding agar sugar and other desired supplement of the final volume of 1 liter then pH is adjust and the medium is autoclaved. Use of powder medium saves time and also money but in experimental works change in the organic and inorganic constituent in the medium are required quantitatively and qualitatively. So, we prepare a series of conc. stock solution of MS medium as given in Table - 1.

Requirements

Weighing balance, pH meter, NaOH, HCl, beakers, glass rod, Distilled water, water bath, autoclave, agar, sugar, various salts given in Table -1.

Stock solution

Four stock solution (Table -1) marked as A, B, C and D were prepared by dissolving the required chemicals in the distilled water as given in Table - 1. To avoid precipitation, dissolve one component at a time. Dissolving of inorganic nitrogen source of the major salt first will avoid precipitation between potassium and calcium source when added subsequently can occur, the pH approaches 6.0, dissolving the calcium separately before adding it will also help to avoid precipitation usually stock solution are prepared in the 10X, 100X and 200X concentration. The stock solution consists of major salt, minor salt, organic nutrient except sucrose. All the stock

solution is stored in proper plastic or glass bottle under refrigeration.

Procedure

1. Recommended quantity of agar and sucrose are weight and dissolved in water. The final volume of the medium by heating them in a water bath or at low pressure.
2. Appropriate quantities of the various stock solutions (Table -2) including growth regulator (if any) are added.
3. The final volume of medium is medium is made up with D.W.
4. After mixing well. The pH of medium is adjusted using 0.1 N NaOH and 0.1 NH_4Cl.
5. The medium is poured into desired culture vessels (about 15 ml in 25x 25 mm culture tube).
6. The culture vessels are plugged with non absorbent cotton wool wrapped in cheese cloth or any other suitable closure.
7. The culture vessel are transparent to the autoclave covered them with aluminum fail to check wetting of the plug while autoclaving at 120^0C for 15 min.
8. The medium is then allowed to coat at room temperature and kept the culture tube in slanting position while cooling in culture room. Slanting provides larger surface area for tissue growth. It is also easier to photograph culture growth on such slants.

Table 1: Composition of Murashige and Skoog (1962) medium

Stock solutions	Chemical formula	Chemical compounds	Concentration in Stock solution (mg/l)	Final concentration in medium (mg/l)
A. Macrosalts (10X)	KNO_3	Potassium nitrate	19000.00	1900.000
	NH_4NO_3	Ammonium nitrate	16500. 00	1650.000
	$CaCl_2.2H_2o$	Calcium chloride	4400. 00	440.000
	$MgSo_4.7H_2o$	Magnesium sulphate	3700. 00	370.000
	KH_2PO_4	Potassium dihydrogen orthophosphate	1700. 00	170.000

B. Microsalts (100X)	$MnSO_4.4H_2O$	Manganese(II) Sulfate Tetrahydrate	2230. 00	22.300
	$ZnSO_4.7H_2O$	Zinc Sulfate Heptahydrate	8600. 00	8.600
	H_3BO_3	Boric Acid	6200. 00	6.200
	KI	Potassium iodide	830. 00	0.830
	$NaMoO_4.2H_2O$	Sodium molybdate dehydrate	250. 00	0.250
	$CuSO_4.5H_2O$	Copper (II) Sulfate Pentahydrate.	25. 00	0.025
	$CoCl_2.6H_2O$	Cobalt(II) Chloride Hexahydrate.	25. 00	0.025
C. Chelating iron (200X)	$Na_2EDTA.2H_2O$	Disodium ethylenediaminetetraacetate dihydrate	5560. 00	37.300
	$FeSO_4.7H_2O$	Iron (II) Sulfate Heptahydrate	7460. 00	27.300
D. Organic components (100X)	$C_6H_{12}O_6$	Myo-inositol	10000. 00	100.000
	$C_2H_5NO_2$	Glycine	200. 00	2.000
	$C_6H_5NO_2$	Nicotinic acid	50. 00	0.500
	$C_8H_{11}NO_3.HCl$	Pyridoxin-HCl (Vit. B_6)	50. 00	0.500
	$N_{12}H_{17}CIN_4.5HCl$	Thiamine-HCl (Vit. B_1)	10. 00	0.100

Table 2: Amount of different stock solution required to prepare one liter of medium.

Stock Solution	MS Full Strength	MS Half Strength
1. Macrosalts (10X)	100 ml	50 ml
2. Microsalts (100X)	10 ml	5 ml
3. Chelating agent (200X)	5 ml	2.5 ml
4. Organic components (100X)	10 ml	5 ml
5. Sugar	30 gm	30gm

Precautions

1. Agar should be properly mixed and any lump formation due to agar be checked.
2. Stock solution prepared processes be carried out in clean or aseptic condition to minimize the chances of microbial contamination.
3. pH of the medium should be accurately maintained, otherwise medium may not settle down.
4. The salts should be measured accurately.