B1163 CRYSTAL VIOLET PECTATE MEDIUM

Formula

Ingredients: gms/lit.

Sodium polypectate	18.00
Sodium hydroxide	0.36
Sodium nitrate	2.00
Calcium chloride.H2O	0.60
Crystal violet	0.0015
Sodium lauryl sulphate	0.10
Agar	4.00

Final pH (at 25°C): 7.2± 0.2

Directions:

Suspend 24.96 grams of dehydrated medium in 1000 ml distilled water. Place on magnetic stirrer with no heat. While stirring, ensure each particle is wetted. When all particles are uniformly wetted in suspension, turn heater on and bring to almost boiling state with continuous mixing. While hot, check pH and adjust, if necessary, with 1M NaOH. (Add NaOH drop by drop and do not overshoot). Heat to boiling to dissolve the medium completely. Cap the flask with aluminum foil rather than cotton plug. Sterilize by autoclaving at 15 lbs pressure (121°C) for 25 minutes. Avoid foaming and pour into Petri plates as soon as possible while hot, (at 50°C) since the medium solidifies quickly and cannot be remelted. Streak or spot inoculate on plates. Note: The surface of agar should be completely dry prior to use.

Principle:

This medium contains crystal violet, which makes it selective for gram-negative bacteria and prevents growth of unwanted organisms. Polypectate serves as suitable source of carbon for polypectate utilizers. Production of enzyme by a culture on this medium is detected either by observing depressions in the gel around the colony where the substrate has degraded, or by flooding the plate with a precipitant solution (1% aqueous solution of hexadecyl trimethyl ammonium bromide can be used as a precipitant). A clear zone will appear around producer colonies where the substrate has degraded and thus precipitation does not occur, while non-producing colonies will be surrounded by opaque gel containing the non-degraded pectin or pectate substrate. Alternately, to study enzyme activity, holes can be made in agar gel plates with a cork borer in order to assay liquid samples, such as culture filtrates. This is referred as well plate or cup plate technique for enzyme assays.

QC Tests – (I)Dehydrated Medium			
	Colour:	Cream to yellow	
	Appearance:	Homogeneous Free Flowing powder	
(II)Rehydrated medium			
	pH (post autoclaving/heating):	7.2 ± 0.2	
	Colour (post autoclaving/heating):	Bluish grey	
	Clarity (post autoclaving/heating):	Clear to slightly opalescent	
(III)Q.C. Test Microbiological			
	Cultural characteristics observed after an incubation at 35-37°C for 72 hours		
	MICROORGANISM (ATCC)	Growth	Liquefaction / sinking of colonies
	Erwinia carotovora (15713)	Luxuriant	positive reaction
	Erwinia chrysanthemi (11663)	Luxuriant	positive reaction

Precautions :	1. For Laboratory Use.
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.
Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

Refer disclaimer Overleaf

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Use:	It is recommended for the cultivation of pectolytic microorganisms, which can degrade sodium polypectate in the medium.				
Storage :	Dehydrated medium- below 30°C Prepared medium– Between 2 to 8°C.				
Packing :	500 gm bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
B1163	24.96 g/l	20.032 L	7.2 ± 0.2	Nil	121^0C / 25 minutes