Mycoplasma Supplement-G

PRODUCT INFORMATION

P028-10MU - Penicillin G Potassium, Powder, 10MU

P028-25MU - Penicillin G Potassium, Powder, 25MU

P028-100MU - Penicillin G Potassium, Powder, 100MU

P006-1MU - Penicillin G Sodium, Powder, 1MU

P006-10MU - Penicillin G Sodium, Powder, 10MU

P006-25MU - Penicillin G Sodium, Powder,25MU

P006-100MU - Penicillin G Sodium, Powder, 100MU

DESCRIPTION

Mycoplasma Agar Base with Mycoplasma Supplement-G is a basic medium which will support the growth of *Mycoplasma* species.

Mycoplasma Agar Broth with Mycoplasma Supplement-G is a basic medium which can be used in the isolation and cultivation of mycoplasmas from clinical specimens.

BACKGROUND

Yeast extract is the common name for various forms of processed yeast products made by extracting the cell contents (removing the cell walls); they are used as food additives or flavourings, or as nutrients for bacterial culture media.

Thallous acetate is a salt of thallium and acetate. It is used in microbiology as a selective growth medium.

Penicillin is a group of antibiotics derived from Penicillium fungi. All penicillins are beta-lactam antibiotics and are used against Gram-positive organism.

Mechanism of action

Bacteria constantly remodel their peptidoglycan cell walls, simultaneously building and breaking down portions of the cell wall as they grow and divide. β-Lactam antibiotics work by inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall. The β-lactam moiety (functional group) of penicillin binds to the enzyme (DD-transpeptidase) that links the peptidoglycan molecules in bacteria. The enzymes that hydrolyze the peptidoglycan cross-links continue to function, which weakens the cell wall of the bacterium (in other words, the antibiotic causes cytolysis or death due to osmotic pressure). In addition, the build-up of peptidoglycan precursors triggers the activation of bacterial cell wall hydrolases and autolysins, which further digest the bacteria's existing peptidoglycan.

APPLICATION IN MYCOPLASMA AGAR BASE

Mycoplasma Agar Base was formulated as a basal medium to be enriched with any satisfactory supplementary factors used for the growth of mycoplasmas (PPLO).

Edward stressed the importance of the absence of toxic factors to mycoplasmas in the basal medium. Lynn & Morton paid special attention to the inhibitory factors which can be present in batches of agar. Mycoplasma Agar Base contains selected constituents shown to be free from such inhibitory or toxic substances. It also contains a special mineral supplement which improves the growth and colony characteristics of mycoplasmas without interfering with the clarity of the medium.

Hayflick suggested inclusion of 10% v/v of a 25% w/v extract of baker's yeast in the medium and Lemcke used yeast extract. The majority of mycoplasmas require media enriched with serum; horse serum (20% v/v) is commonly used. Swine or human sera may be substituted for horse serum but the possible presence of antibodies or antibiotics in human serum make media control of great importance (Fallon). The addition of DNA to the medium to encourage the growth of bovine general genital strains and other mycoplasmas was suggested by Edward. 20 mg of sodium deoxyribonucleate per ml of medium is quoted by Lemcke.

Antibacterial agents are necessary to prevent overgrowth of the slow-growing mycoplasmas by contaminating organisms. Penicillin and thallous acetate are the most common agents used but *Ureaplasma urealyticum* are

sensitive to thallous acetate. Hutchinson and Fallon state that ampicillin at 1 mg/ml of medium may be substituted for penicillin and thallous acetate.

Penicillin may be used at concentrations between 50 and 500 units per ml and thallous acetate between 1/2000 and 1/8000 (Lemcke). It is preferable to omit thallous acetate when searching for *Ureaplasma urealyticum* (Shepherd & Lanceford).

Two supplements, Mycoplasma Supplement-G and Mycoplasma Supplement-P have been developed for the improved growth of mycoplasmas. Mycoplasma Supplement-G is a general supplement prepared to the formulation of Hayflick which, when added to Mycoplasma Broth or Agar Base produces a complete selective medium for the propagation of sterol-requiring Mycoplasma species of the classical type.

Mycoplasma Supplement-P is a liquid supplement based on the formulation recommended by the Mycoplasma Reference Laboratory, CPHLS, Colindale, which is used in conjunction with Mycoplasma Agar Base to form a bi-phasic medium for the isolation and preliminary identification of *Mycoplasma pneumoniae*.

Many species of mycoplasmas are aerobes or facultative anaerobes but some prefer micro-aerophilic conditions with the addition of carbon dioxide, or strict anaerobiosis.

Pathogenic strains grow best at 35°C while saprophytic strains often grow between 22°C and 30°C, *Ureaplasma urealyticum* have an optimal temperature of 36°C.

Mycoplasma species grow best at pH 7.4-8.0 but *Ureaplasma urealyticum* prefer pH 6.0-6.5.

Content concentrations

Typical Formula*	**mg/litre**
Mycoplasma Agar Base	
Bacteriological peptone	10
`Lab-Lemco' powder	10
Sodium chloride	5
Mineral supplement	0.5
Agar	10
Final pH 7.8 ± 0.2 @ 25°C	
Mycoplasma Supplement-G	
Horse serum	200.0 ml
Yeast extract (25% w/v)	100.0 ml
Thallous acetate	250
Penicillin	200,000 IU
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Mycoplasma Agar Base and Mycoplasma Supplement-G

METHOD

Preparation

Add appreciate mount of Mycoplasma Agar Base to distilled water. Boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and add Mycoplasma Supplement-G reconstituted as directed.

Protocol

Agar plates

Material for cultivation is inoculated onto agar plates (usually 55mm) prepared with Mycoplasma Agar Base + Mycoplasma Supplement-G. Plates are incubated in moist chambers aerobically, anaerobically and in 10% CO2-90% N2 atmosphere.Examine the agar surface after 7 days incubation with a dissecting microscope at 60x magnification, using obliquely transmitted light. The colonies are characteristic with the centre of the colony embedded beneath the surface, giving a `fried-egg' appearance.

Purification of the organism by further cloning subcultures is essential before identification. This may be carried out by removing a plug of agar containing a colony from the plate and using it to inoculate further plates of medium. Growth inhibition tests using specific antisera may then be carried out (Clyde).

BI-phasic Medium

Bi-phasic media prepared with 1ml quantities of solid Mycoplasma Agar Base overlaid with 2 ml of reconstituted Mycoplasma Supplement-P SR0060. Bi-phasic medium bottles should be inoculated with a swab or a fleck of sputum and incubated at 35°C for up to three months. Any bottles showing gross turbidity due to growth of bacteria or fungi should be discarded.

Growth of *Mycoplasma pneumoniae* results in the reduction of methylene blue followed by production of acid due to the fermentation of glucose, resulting in a colour change of the phenol red indicator to yellow. Bottles showing such a colour change should be sub-cultured onto agar for further examination. Mycoplasma Agar Base supplemented with Mycoplasma Supplement-G is suitable for this purpose.

Quality control

Positive control:

Mycoplasma pneumoniae ATCC® 15531: Microscopic examination- 'fried-egg' colonies

Negative control:

Escherichia coli ATCC® 25922: Inhibited

REFERENCES

1. Edward D. G. ff. (1971) J. Gen. Microbiol. 69. 205-210.

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3. Hayflick L. (1965) Texas Rep. Biol. & Med. 23. suppl. 1. 285-303.

4. Lemcke Ruth M. (1965) `Media for the Mycoplasmataceae', Lab. Pract. 14. 712.

5. Hutchinson D. (1969) J. Med. Lab. Technol. 26. 111-116.

6. Fallon R. J. (1969) S. A. B. Technical series 3. Academic Press. 41-50.

7. Edward D. G. ff. (1954) J. Gen. Microbiol. 10. 27-64.

8. Shepard M. C. and Lanceford C. D. (1970) Appl. Microbiol. 2. 539-543.

9. Clyde W. A. (1964) J. Immun. 92. 958-962

APPLICATION IN MYCOPLASMA BROTH BASE

Mycoplasma Broth Base complements Mycoplasma Agar Base.

It requires supplementation with yeast extract, serum and antibiotics, which are available as Mycoplasma Supplement-G.

Carbohydrate fermentation by mycoplasmas can be tested by incorporating 1% w/v carbohydrate and 0.005% w/v phenol red into the broth medium.

A Ureaplasma broth can be prepared by adding to 95 ml broth medium (pH adjusted to 6.0):

Contents	
Horse Serum	4.0ml
Urea	0.05g
Phenol red	0.001g
Penicillin	100,000 units

A similar medium was described by Taylor-Robinson et al. where reference is made to the use of HEPES buffer to induce large colony-forming Ureaplasma strains and for the isolation and titration of viable mycoplasma by the metabolism of arginine or glucose and measuring the pH change in the medium.

A selective medium for Mycoplasma pneumoniae was described by Kraybill & Crawford.

Most strains of mycoplasmas are encouraged by growth in a biphasic medium in which a layer of Mycoplasma Broth Base covers a basal layer of Mycoplasma Agar Base. Both broth and agar layers should be supplemented with Mycoplasma Supplement-G. Inclusion of the supplement provides the necessary factors for growth and prevents overgrowth of slow growing contaminating organisms.

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Protocol

Quality control

Positive control:

Mycoplasma pneumoniae ATCC® 15531: Slightly turbidNegative control:

Negative control:

Escherichia coli ATCC® 25922: Inhibited

REFERENCES

1. Shepard M. C. and Lanceford C. D. (1970) Appl. Microbiol. 20. 539-543.

2. Taylor-Robinson D., Martin-Bourgon C., Watanable T. and Addey J. P. (1971) J. Gen. Microbiol. 68. 97-107.

3. Kraybill W. H. and Crawford Y. E. (1965) Proc. Soc. Exp. Biol. Med. 118. 965-967.