7.13 Experimental Microbial Genetics

Fall 2008

MUCOID COLONY ASSAY

P. aeruginosa strains isolated from CF patients with advanced infections often display the mucoid morphotype. Due to an overproduction of the polysaccharide alginate, these mucoid colonies are extremely *glossy* and *slimy*. By facilitating adherence and restricting access to leukocytes and antibiotics, the mucoid conversion is thought to significantly contribute to CF mortality.

1) Pour *Pseudomonas* Isolation Agar (PIA) plates
   For 1L of plates:
   - 45 g PIA powder
   - 20 mL glycerol
   - 1 L distilled water

   Autoclave 30 min, let agar cool in 50°C waterbath, pour plates, let sit on bench O/N to solidify and dry
2) Plate/streak strain of interest on PIA
3) Incubate colonies 2-4 days at 37°C
4) Observe colonies for mucoid morphology (shiny! gooey!)

More Info on PIA:

Pseudomonas Isolation Agar

Intended Use

Pseudomonas Isolation Agar is used with added glycerol in isolating *Pseudomonas* and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pigment formation.

Summary and Explanation

Pseudomonas aeruginosa is an opportunistic pathogen that can infect eyes, ears, burns and wounds.[1] It is also a leading cause of hospital acquired infections. Patients undergoing antibiotic therapy are especially susceptible to infection by *Pseudomonas aeruginosa*.

Pseudomonas Isolation Agar is prepared according to a slight modification of the Medium A formulation of King, Ward and Raney.[2] Pseudomonas Isolation Agar includes Irgasan™, a potent broad spectrum antimicrobial that is not active against *Pseudomonas*.[3] As well as being selective, Pseudomonas Isolation Agar is formulated to enhance the formation of the blue or blue-green pyocyanin pigment by *Pseudomonas aeruginosa*. The pigment diffuses into the medium surrounding growth.

Irgasan™ is a trademark of Ciba-Geigy

Principles of the Procedure

Peptone provides the carbon and nitrogen necessary for bacterial growth. Magnesium chloride and potassium sulfate promote production of pyocyanin. Irgasan, an antimicrobial agent, selectively inhibits gram-positive and gram-negative bacteria other than *Pseudomonas* spp. Agar is the solidifying agent. Glycerol serves as an energy source and also helps to promote pyocyanin production.

User Quality Control

Identity Specifications

Difco™ Pseudomonas Isolation Agar

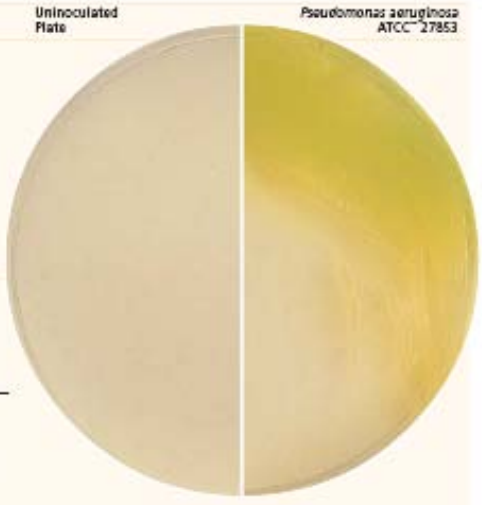
Dehydrated Appearance:	Very light beige, homogeneous, free-flowing.
Solution:	4.5% solution, soluble in purified water containing 2% glycerol upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Pseudomonas Isolation Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	APPEARANCE
Escherichia coli	25922	10^3-2 x 10^3	Marked to complete inhibition	–
Pseudomonas aeruginosa	10145	10^2-10^3	Good	Green to blue-green
Pseudomonas aeruginosa	27853	10^2-10^3	Good	Green to blue-green



Formula

Difco™ Pseudomonas Isolation Agar

Approximate Formula* Per Liter

Peptone	20.0	g
Magnesium Chloride	1.4	g
Potassium Sulfate	10.0	g
Irgasan™	25.0	mg
Agar	13.6	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water containing 20 mL of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the medium using the streak plate method to obtain isolated colonies. Incubate for 18-48 hours at 35 ± 2°C.

Expected Results

Examine for the presence of good growth. *Pseudomonas aeruginosa* colonies may be greenish after incubation for 18 hours and turn blue to blue-green as incubation continues up to 24-48 hours, with diffusion of the pigment into the medium.

Limitations of the Procedure

1. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.[3,4]
2. Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*. Consult appropriate references.[1,5]

References

1. Klein and Gilligan. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. King, Ward and Raney. 1954. J. Lab. Clin. Med. 44:301.
3. Paris and Schonlet. January, 1948. Soap and Chemical Specialties.
4. Gaby and Fett. 1931. J. Bacteriol. 22:349.
5. Pezzlo. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Pseudomonas Isolation Agar

Cat. No.	292710	Dehydrated – 500 g
Europe		
Cat. No.	257002	Prepared Plates – Pkg. of 20*
Mexico		
Cat. No.	252648	Prepared Plates (90 × 15 mm-style) – Pkg. of 20*

Difco™ Glycerol

Cat. No.	228210	Bottle – 100 g
	228220	Bottle – 500 g

*Store at 2-8°C