ISOLATION AND PRESUMPTIVE IDENTIFICATION OF *FUSOBACTERIUM NECROPHORUM* FROM THROAT SWABS

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Abstract

Studies have shown that *Fusobacterium necrophorum* could be one of the leading causes of acute tonsillitis and peritonsillar abscess. This has led to a renewed interest in performing throat cultures anaerobically for the isolation of *F. necrophorum*, as well as a need for a media designed for isolation and presumptive identification of the organism. A PRAS Egg Yolk Agar was used as the base for our identification media due to the presence of a lipase reaction with *F. necrophorum*. 1.0mL/L of vancomycin was added as well kanamycin in concentrations of 1.0mL/L, 2.0mL/L, and 4.0mL/L. All 3 formulations were tested with *Fusobacterium necrophorum* (ATCC 25286), *Fusobacterium nucleatum* (ATCC 25586), *Clostridium perfringens* (ATCC 13124), *Bacteroides fragilis* (ATCC 25285), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 7002), and *Staphylococcus aureus* (ATCC 25923). At 1.0mL/L of Kanamycin 3rd and 4th quadrant growth was observed in all organisms with the exception of *S. aureus* which exhibited no growth. At 2.0mL/L inhibition of *F. nucleatum* was observed along with the lack of growth from *S. aureus*. All other organisms exhibited strong growth into the 4th quadrant. At 4.0mL/L all organisms were inhibited with the exception of *F. necrophorum* and *B. fragilis*. Only *F. necrophorum* exhibited a lipase reaction. Based on this study, a PRAS Egg Yolk Agar containing 1.0mL/L vancomycin and 4.0mL/L kanamycin (EYKV) will aid in isolating and providing presumptive identification of *Fusobacterium necrophorum* within 24 to 48 hours of acquiring an acceptable anaerobic specimen.

Introduction

To determine the optimum media for isolation and presumptive identification of *Fusobacterium necrophorum*, 3 different formulations were tested using varying concentrations of kanamycin. Starting with a PRAS egg yolk agar in order to facilitate a lipase reaction, 7.5mg/L vancomycin was added to inhibit Gram positive organisms. Kanamycin was added in concentrations of 100.0mg/L, 200.0mg/L and 400.0mg/L in order to inhibit facultative and Gram negative organisms.

Objective

Determine the optimum media formulation for the isolation and presumptive identification of *Fusobacterium necrophorum*.

Materials and Methods

Organisms: The organisms used for this study were obtained from the American Type Culture Collection (ATCC, Bethesda, MD). Strains tested were: *Fusobacterium necrophorum 25286, Bacteroides fragilis 25285, Prevotella melaninogenica 25845, Fusobacterium nucleatum 25586, Clostridium perfringens 13124, Clostridium difficile 9689, Peptostreptococcus anaerobius 27337, Proteus mirabilis 7002, Escherichia coli 25922, and Enterococcus faecalis 29212*. The organisms were subcultured from -70°C freezer stocks onto pre-reduced Brucella Blood Agar (BRU) plates (Anaerobe Systems, Morgan Hill, CA) before inoculation on to each agar formulation. All manipulations of bacteria were performed in an AS-580 anaerobic chamber (Anaerobe Systems, Morgan Hill, CA).

Inoculation: A solution with turbidity equivalent to a 0.5 McFarland was created in 1.0 mL PRAS saline blanks (Anaerobe Systems). Agar formulations were inoculated with 10µL of standardized inoculum from 24 hour old Brucella Blood Agar (BRU, AS-111, Anaerobe Systems). All manipulations of bacteria were performed under anaerobic conditions.

Media: The media used for this study were Pre-Reduced Anaerobically Sterilized (PRAS) agar formulations (See below). Sixteen grams of each formulation was dispensed under anaerobic conditions into 15mmX100mm culture dishes. Plates were packaged in foil pouches under anaerobic conditions and stored at room temperature.

Formula

Pancreatic Digest of Casein	20.0 g
Yeast Extract	5.0 g
Pyruvate	0.5 g
Sodium Chloride	2.5 g
Dextrose	2.0 g
L-Tryptophan	0.2 g
Tween 80	1.0 ml
Hemin (0.1% soln)	5.0 ml
Vitamin K1 (1% soln)	1.0 ml
L-Cystine	0.4 g
Sodium Phosphate	5.0 g
Magnesium sulfate (5% soln)	0.2 ml
Agar	20.0 g
Egg Yolk Suspension	100.0 ml
Kanamycin	100.0 mg
	200.0 mg
	400.0 mg
Vancomycin	7.5 mg
Distilled Water	1000.0 ml

Final pH 7.1 +/- 0.2 at 25 degrees C.
Final weight 16.0 g +/- 1.6.

Incubation: All media formulations were incubated at 37°C in an AS-580 anaerobic chamber.

Reading: Growth for each organism on each formulation was recorded after 24 and 48 hours of incubation. The presence of colonies in both the 3rd and 4th quadrant was recorded as Growth. Growth in the 1st and 2nd quadrant with no growth in the 3rd or 4th was recorded as Inhibited Growth. No colony formation is recorded as No Growth.

***Fusobacterium necrophorum*, 24 hour growth on Egg Yolk Agar with 7.5mg/L vancomycin and 400mg/L kanamycin:**

***Bacteroides fragilis*, 24 hour growth on Egg Yolk Agar with 7.5mg/L vancomycin and 400mg/L kanamycin:**

***Fusobacterium necrophorum*, 48 hour growth on Egg Yolk Agar with 7.5mg/L vancomycin and 400mg/L kanamycin:**

***Bacteroides fragilis*, 48 hour growth on Egg Yolk Agar with 7.5mg/L vancomycin and 400mg/L kanamycin:**

***Fusobacterium necrophorum* colonies, 48 hour growth on Egg Yolk Agar with 7.5mg/L vancomycin and 400mg/L kanamycin:**

***Bacteroides fragilis* colonies, 48 hour growth on Egg Yolk Agar with 7.5mg/L vancomycin and 400mg/L kanamycin:**

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Results

At 100.0mg/L kanamycin, no growth was observed with *Clostridium perfringens, Clostridium difficile, Peptostreptococcus anaerobius*, and *Enterococcus faecalis*. Growth was observed in all other organisms within 24 hours at 100.0mg/L kanamycin with Fusobacterium necrophorum exhibiting a low level of lipase activity within 24 hours and strong level within 48 hours. At 200.0mg/L kanamycin both *Fusobacterium nucleatum* and *Escherichia coli* exhibited inhibited growth. All other organisms remained the same. At 400.0mg/L only *Fusobacterium necrophorum, Bacteroides fragilis*, and *Prevotella melaninogenica* grew within 24 hours. Only *Fusobacterium necrophorum* exhibited lipase activity. (See Table 1)

Table 1: Organism growth per concentration of kanamycin

	100.0mg/L	200.0mg/L	400.0mg/L
Fusobacterium necrophorum (Lipase positive at all concentrations)	+	+	+
Bacteroides fragilis	+	+	+
Prevotella melaninogenica	+	+	+
Fusobacterium nucleatum	+	+/ -	-
Clostridium perfringens	-	-	-
Clostridium difficile	-	-	-
Peptostreptococcu anaerobius	-	-	-
Proteus mirabilis	+	+	-
Escherichia coli	+	+/ -	-
Enterococcus faecalis	-	-	-

+ Growth, - No Growth, +/- Inhibited

Discussion

The ideal formulation for the isolation and presumptive identification of *Fusobacterium necrophorum* was found to be an Egg Yolk Agar containing 7.5mg/L vancomycin and 400.0mg/L kanamycin. With this formulation, growth combined with lipase activity can be used to presumptively identify *F. necrophorum*. In order to positively identify the organism, this should be combined with spot indole as well as gram stain. *Fusobacterium necrophorum* will present with a positive indole reaction, and characteristic gram negative rods.

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