AEROMEDICAL REVIEWS

A SINGLE CULTURE MEDIUM FOR THE DIFFERENTIATION AND IDENTIFICATION OF THE GRAM-NEGATIVE, NONFERMENTATIVE BACILLI OF HOSPITAL IMPORTANCE

Review 1-63

USAF SCHOOL OF AEROSPACE MEDICINE
AEROSPACE MEDICAL DIVISION (AFSC)
BROOKS AIR FORCE BASE, TEXAS

A SINGLE CULTURE MEDIUM FOR THE DIFFERENTIATION AND IDENTIFICATION OF THE GRAM-NEGATIVE, NONFERMENTATIVE BACILLI OF HOSPITAL IMPORTANCE

WALTER M. SELLERS, JR.

Astrobiology Branch

Review 1-63

USAF SCHOOL OF AEROSPACE MEDICINE
AEROSPACE MEDICAL DIVISION (AFSC)
BROOKS AIR FORCE BASE, TEXAS

March 1963

A SINGLE CULTURE MEDIUM FOR THE DIFFERENTIATION AND IDENTIFICATION OF THE GRAM-NEGATIVE, NONFERMENTATIVE BACILLI OF HOSPITAL IMPORTANCE

The group of nonfermenting, gram-negative bacilli are considered by many diagnostic bacteriologists to be the most difficult organisms to classify in the clinical laboratory because they produce no distinctive characteristics on the standard culture media. This group is composed of the following organisms: *Pseudomonas aeruginosa*, *Bacterium anitratum*, *Herellea vaginicola*, *Mima polymorpha*, and *Alcaligenes faecalis*. All are pathogenic except the last. The nonpathogen is of importance, however, because of its close resemblance to the pathogens. Because of their similar appearance on routine differential media, these organisms can only be differentiated from one another by time-consuming approaches requiring a number of special differential media and biochemical tests. To date, no single differential medium has been available for the identification of the members of this group of organisms.

This report describes a culture medium which will identify each of these five similar organisms. In addition, it is more reliable for the identification of *Ps. aeruginosa* than is either of the two commercial media used for this purpose, because it is not necessary to depend on a single parameter (pyocyanine or fluorescin) for the identification when it is used. Basic differences in growth characteristics which were used in the development of the medium are outlined in table I. Composition of the medium is shown in table II.

MATERIALS AND METHODS

The ingredients are dissolved in distilled water with the magnesium sulfate and the potassium phosphate dissolved sep-

TABLE I

Physiologic differences

	Ps. aeruginosa	*A. faecalis*	*M. polymorpha*	*H. vaginicola*	*B. anitratum*
Obligate aerobic growth in the presence of nitrate	−	−	+	+	+
Glucose oxidized to acid in the presence of high peptone concentrations	−	−	−	+	+
N_2 gas production	+	+	−	−	−
Fluorescence	+	−	−	+	−

TABLE II

Medium composition

Water	—	1,000 ml.
Sodium chloride	—	2.0 gm.
Sodium nitrate	—	1.0 gm.
Sodium nitrite	—	0.35 gm.
D-mannitol	—	2.0 gm.
L-arginine	—	1.0 gm.
Yeast extract	—	1.0 gm.
$MgSO_4 \cdot 7H_2O$	—	1.5 gm.
$K_2HPO_4 \cdot 3H_2O$	—	1.0 gm.
Bacto peptone	—	20.0 gm.
Bromothymol blue	—	0.04 gm.
Phenol red	—	0.008 gm.
Bacto agar	—	15.0 gm.

Adjusted to pH ± 7.

arately in small amounts of water before adding them to the medium, thereby avoiding the formation of an insoluble magnesium phosphate precipitate. Stock solutions of the indicator dyes are dissolved in 0.01 normal NaOH at a concentration of 4 mg. per milliliter for the bromothymol blue and 2 mg. per milliliter for the phenol red. The medium is boiled to dissolve the agar and tubed in 10 ml. quantities. The tubes are autoclaved for 10 minutes at 15-pound pressure (121° C.) and slanted to make 1½-inch butts and 3-inch slants. Immediately before inoculating, approximately 0.15 ml. of a sterile 50% glucose solution is added per tube (2 drops from a 10 ml. pipet). The butt is then stabbed and the slant streaked according to the usual procedure. The inoculated tubes are incubated at 37° C. for 24 hours.

RESULTS

The reactions of each of the nonfermenting organisms on the medium are given in table III.

TABLE III

Characteristic reactions on medium

Organism	Color of slant	Color of surface growth	Yellow band at junction of slant and butt	N_2 gas production	Greenish yellow fluorescent slant	Fluorescent band at junction of butt and slant	Color of butt
Ps. aeruginosa	Olive	Orange	Negative	Positive	Positive	Negative	Blue
H. vaginicola	Blue	White	Positive	Negative	Negative	Positive	Green
B. anitratum (typical strains)	Blue	White	Positive	Negative	Negative	Negative	Green
B. anitratum (atypical strains)	Yellow	White	Positive	Negative	Negative	Negative	Green
M. polymorpha	Blue	White	Negative	Negative	Negative	Negative	Green
A. faecalis	Blue	White	Negative	Positive	Negative	Negative	Blue

Uninoculated medium is green.

DISCUSSION

Although several of the components of the medium could be varied both quantitatively and qualitatively and still allow results similar to those given in table II, the recommended combination produces the best balanced medium.

Fermenting organisms are easily distinguished from the group of nonfermenting bacteria. Even such nonmannitol fermenting organisms as *Proteus vulgaris* and *P. mirabilis* will produce acid (yellow) butts. This is due to the small amount of added glucose transmitted to the butt by the stab inoculation and by diffusion during the incubation period.

The small amount of glucose added to the culture tubes immediately before inoculation allows *B. anitratum* and *H. vaginicola*, both of which are glucose oxidizers, to produce an acid (yellow) band at the junction of the slant and butt. The glucose also stimulates *H. vaginicola* to produce a band of fluorescence immediately above the yellow band. *Ps. aeruginosa*, a very proteolytic organism, fails to produce the yellow band because of the arginine and high peptone concentration, even though it will produce an acid reaction from glucose under other conditions.

M. polymorpha, *H. vaginicola*, and *B. anitratum* are obligate aerobes which are unable to grow anaerobically even in the presence of nitrate. They are incapable of growing in the butt of the tubes and therefore the pH indicator remains unchanged. *Ps. aeruginosa*, although it is an obligate aerobe under strict conditions, is able to grow in the butt of this medium because it will grow anaerobically in the presence of nitrate. It produces an alkaline reaction in the butt as does *A. faecalis*, a facultative anaerobe.

Sodium nitrate was used in 0.1% concentration. Although $^3/_{10}$% gave the greatest N_2 production, it interfered with glucose oxidation by *B. anitratum*, and *H. vaginicola;* it also interfered with fermentative reactions by some fermenting organisms (e.g. *Proteus*), and was toxic to a few *Ps. aeruginosa* strains. Sodium nitrite 0.035% was the optimum concentration. Larger quantities

were toxic to all of the organisms while a less quantity was ineffective for N_2 gas formation with a number of *Ps. aeruginosa* strains. Sodium nitrite was used because a few *Ps. aeruginosa* strains can produce N_2 from it but not from sodium nitrate. Nitrogen gas production was ascertained by gas bubbles in the condensate and cracked agar butts which were not acid (i.e., yellow).

Dibasic potassium phosphate stimulates N_2 production. Three tenths of 1% was optimum but 0.1% was used because this concentration allowed adequate N_2 production; had less buffering capacity which allowed a faster pH change in the butt; and did not interfere with the acid band formation at the junction of the butt and slant. In addition, this concentration had less tendency to form a precipitate in the presence of magnesium sulfate.

Magnesium sulfate stimulates fluorescein production. The optimum concentration was 0.15%. Less concentration caused a decrease in fluorescence, while more tended to precipitate the phosphate.

Mannitol was used instead of glucose or citrate because it stimulates fluorescein production, does not interfere with anaerobic growth or N_2 production, and is a good energy source for *Ps. aeruginosa.*

Proper utilization of this medium will be of great value to the diagnostic bacteriologist in identifying the troublesome gram-negative nonfermenting bacilli of medical importance. The medium has been used extensively by the Army Surgical Research Unit at Brooke Army Medical Center for six months.