APPLIED MICROBIOLOGY, July, 1966
Vol. 14, No. 4

Improved Motility Medium

ROBERT J. BALL AND WALTER SELLERS

Aerospace Medical Division, U.S. Air Force School of Aerospace Medicine, Brooks Air Force Base, Texas

Received for publication 1 April 1966

ABSTRACT

BALL, ROBERT J. (U.S. Air Force School of Aerospace Medicine, Brooks Air Force Base, Tex.), AND WALTER SELLERS. Improved motility medium. Appl. Microbiol. **14**:670–673. 1966.—An improved motility medium which permits additional cultural characterization is described. Advantages include maximal motility due to a change in the physical state of the medium from solid to liquid at incubation temperatures, a definitive stab line, preservation of the stab line with nonmotile organisms, and visual delineation of culture motility. In addition, nitrate reduction, nitrogen gas production, and gelatin liquefaction may be demonstrated.

The ideal medium for testing motility of bacteria would be one which: (i) offers no more resistance to motility during the incubation period than would a broth culture; (ii) assures a definitive stab line; (iii) preserves the stab line of inoculation during the incubation period in spite of rough handling; (iv) supports good growth and maximal motility with most fastidious organisms; and (v) clearly indicates the extent of motility.

The culture medium described below, we believe, provides these characteristics. In addition to motility, it permits the determination of nitrate reduction, nitrogen gas formation, and gelatin liquefaction.

MATERIALS AND METHODS

The composition of the medium is shown in Table 1. The ingredients were weighed out in the amounts shown, suspended in 1,000 ml of distilled water, heated to dissolve the agar, sterilized in an autoclave for 15 min at 121 C, and cooled to 60 C before adding 10 ml of a 1% filter-sterilized, aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC; Difco). The sterile 1% TTC was kept as a stock solution in a refrigerator. After the addition of the TTC, the flask was swirled to obtain uniform distribution, and 3-ml amounts were aseptically transferred to sterile tubes (12 by 75 mm). The tubes were incubated overnight for sterility and stored at 5 to 10 C until needed. Final reaction of the medium was *p*H 7.3.

Stab inoculations were made immediately upon removal of the medium from the refrigerator since, in these small amounts, the medium warmed up quickly and no longer provided a tough gel. The medium became liquid at about 24 C. The tubes were examined for evidence of motility after overnight incubation at 35 C.

Additional cultural characteristics were ascertained by the following methods. Gelatin liquefaction was determined by placing the cultures, together with an uninoculated control tube of the medium, in the refrigerator until the control tube solidified while positive test cultures did not. Reduction of nitrate to nitrite was determined by the addition of about 3 drops each of solution A (0.8% sulfanilic acid in 5 N acetic acid) and solution B (0.5% α-napthylamine in 5 N acetic acid) to the chilled culture. Since the red formazan formed from TTC reduction is insoluble, it did not interfere with this test. Zinc dust was added to the negative tubes to test for the presence of nitrate. The appearance of a froth at the surface of the medium indicated nitrogen gas production.

TABLE 1. *Composition of motility medium*[a]

Component	Amt
Gelatin (Difco)	30 g
Agar (Difco)	1 g
Heart Infusion Broth	25 g
K_2HPO_4	2 g
KNO_3	2 g
Distilled water	1,000 ml

[a] Unadjusted *p*H 7.3; autoclaved for 15 min at 15 psi; cooled to 60 C; 10 ml of a 1% solution of TTC added.

RESULTS AND DISCUSSION

Figure 1 shows the reaction of nine organisms on four different motility media after 16 hr of incubation at 35 C. Five of the nine organisms are motile (no. 1 through 5). Tubes in the top row marked A contain the improved medium. The first five tubes in row A appear red, whereas

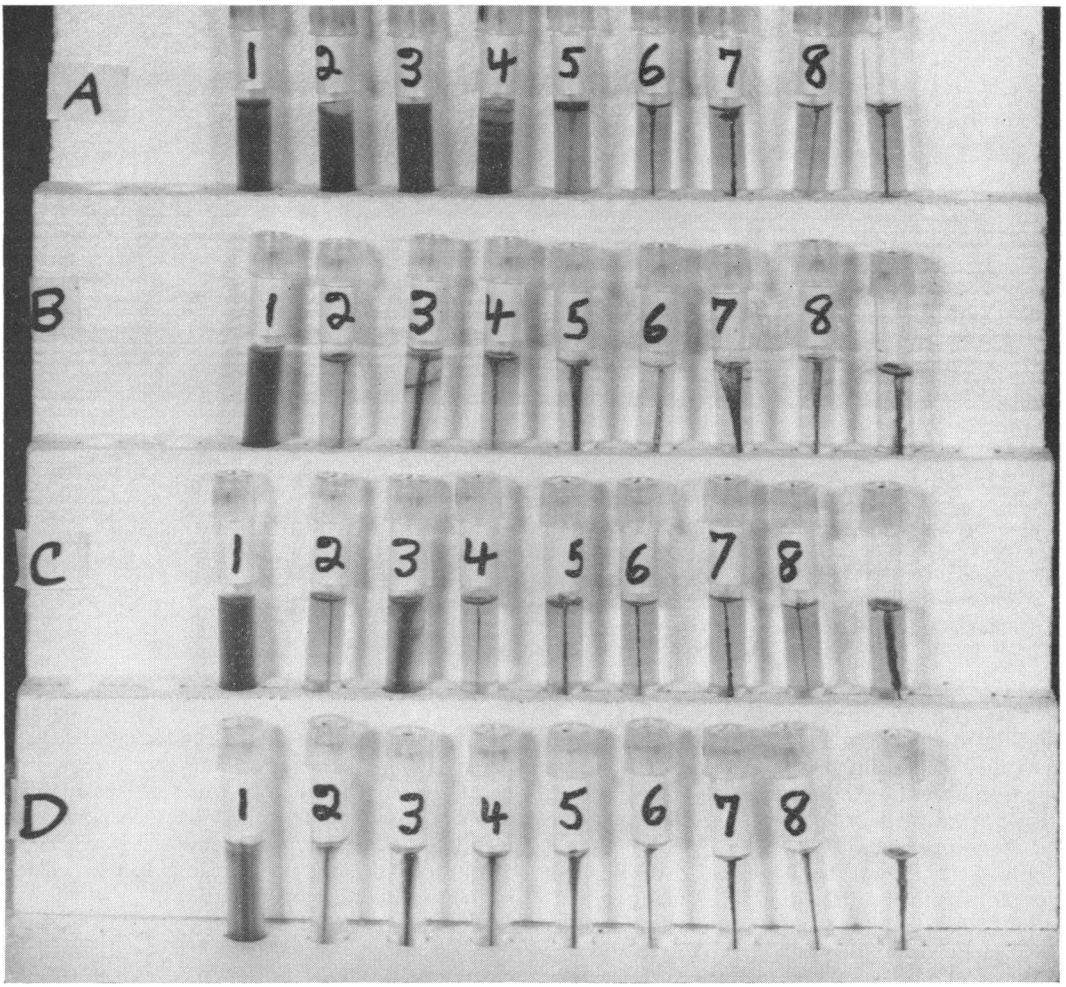

FIG. 1. *Media used: (A) the improved medium; (B) motility medium used at the Communicable Disease Center (E. O. King, unpublished data); (C) GI Motility Medium (Difco); (D) Motility Test Medium (Difco). Organisms used: (1) Salmonella typhimurium; (2) Alcaligenes species; (3) Proteus vulgaris; (4) Pseudomonas aeruginosa; (5) Proteus rettgeri; (6) Mima polymorpha; (7) Alkalescens-Dispar biotype of Escherichia coli; (8) Shigella flexneri; (unmarked tubes) Klebsiella pneumoniae.*

the rest of the tubes are yellow with a red stab line. A comparison of the reactions in row A with those in rows B, C, and D shows that the five motile organisms diffused throughout medium A in 16 hr, but in the other media were generally confined to the stab line except in tube no. 1.

For photographic purposes, TTC was added to the other media in the same concentration as in medium A. Medium B contains 0.4% agar and is used routinely at the U.S. Public Health Service Communicable Disease Center (E. O. King, *unpublished data*). Media C and D contain 0.3 and 0.5% agar, respectively. Media B and D are practically colorless, whereas media A and C are the color of the Heart Infusion base.

Our assays have shown that inhibition of motility varied directly with the agar concentration in our medium, and that 0.1% agar (in the presence of 3% gelatin) was sufficient to preserve an intact stab line with nonmotile organisms in spite of rough handling. It is difficult to obtain a clear-cut stab line in any medium containing less than 0.5% agar (with no gelatin present). For this reason, many bacteriologists stab motility media to a depth of only 5 mm. The cultures in Fig. 1 were stabbed in a routine manner with no added precautions. It can be seen that the stab

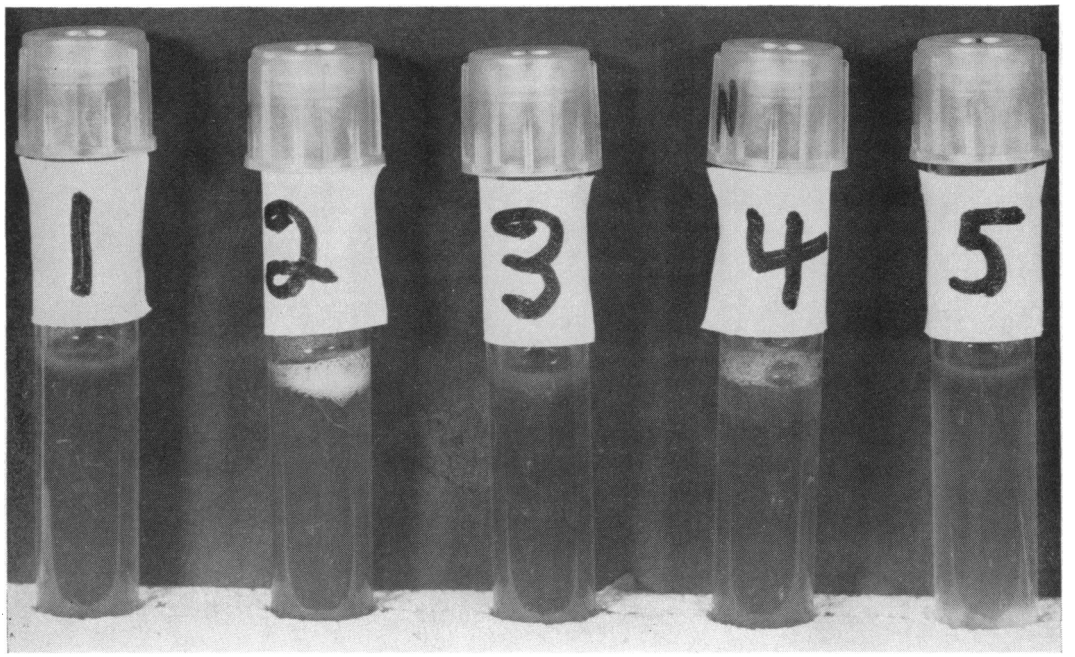

FIG. 2. *Close-up photograph of tubes 1 through 5 in row A of Fig. 1 to show N_2 gas production in tubes 2 and 4.*

lines in row A show less variation than those in the other media. Bubbles seen along stab lines in some cultures are eliminated with medium A, because the medium becomes liquid during the incubation period.

Figure 2 shows the appearance of positive versus negative nitrogen gas-producing organisms. Tubes 2 and 4 have produced nitrogen gas, as shown by the froth at the top of the medium. Cultures were checked for nitrogen gas production after overnight incubation.

Heart Infusion Broth (Difco) was chosen as the basal medium because of its ability to support the growth of fastidious organisms which may fail to grow in ordinary peptone media, and because it is a rich source of calcium which is known to stimulate gelatinase formation (1, 2, 3). Furthermore, it has been reported by a number of investigators (5, 6, 7) that 0.5% sodium chloride, which is the amount contained in the Heart Infusion Broth, induces "swarming" by *Proteus* species. Three per cent gelatin was selected since assays of gelatin concentrations between 1 and 12% indicated that this amount would melt at average room temperatures. Organisms capable of reducing nitrate exhibited increased motility in the presence of 0.2% potassium nitrate. This was especially true of nitrate-reducing obligate aerobes. Dipotassium phosphate was included in the medium because of the fundamental role of phosphate in the intermediary metabolism and because of its stimulatory effect on the motility of *Proteus* strains. The TTC was incorporated to facilitate the detection of motility. Growth along or out from the stab line is readily visible because of the precipitation of formazan (4).

ACKNOWLEDGMENTS

We express our appreciation to Jerome P. Schmidt and Joseph T. Cordaro, Infectious Processes Unit, Microbiology Section, U.S. Air Force School of Aerospace Medicine, Brooks Air Force Base, Tex., for aid received in this investigation.

LITERATURE CITED

1. HAINES, R. B. 1931. The formation of bacterial proteases, especially in synthetic media. Biochem. J. **25**:1851–1859.
2. HAINES, R. B. 1932. The influence of the medium on the production of bacterial gelatinase. Biochem. J. **26**:323–335.
3. HAINES, R. B. 1933. Further studies on the effect of the medium on the production of bacterial gelatinase. Biochem. J. **27**:466–474.
4. KELLY, A. T., AND M. FULTON. 1953. Use of triphenyl tetrazolium chloride in motility test medium. Am. J. Clin. Pathol. **23**:512.
5. NAYLOR, P. G. D. 1960. A simple medium which controls the swarming of *Proteus*. J. Med. Lab. Tech. **17**:182–184.

6. SANDYS, G. H. 1960. A new method of preventing swarming of *Proteus* sp. with a description of a new medium for use in routine laboratory practice. J. Med. Lab. Tech. **17**:224–233.

7. SCHNEIERSON, S. S. 1961. Production of discrete nonswarming colonies by *Proteus* on medium deficient in sodium chloride and other salts. J. Bacteriol. **82**:621–622.