# NOTES

## SELECTIVE MEDIUM FOR FUSOBACTERIA AND LEPTOTRICHIA

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The media of Baird-Parker (Nature **180**:1056, 1957), Onisi (J. Dental Res. **38**:311, 1959), and Omata and Disraely (J. Bacteriol. **72**:677, 1956) contain crystal violet or streptomycin, or both, as inhibitory agents for the selection of fusobacteria and leptotrichia from oral material. De Araujo and Gibbons (J. Bacteriol. **84**:593, 1962) have shown that crystal violet, in the medium of Omata and Disraely, is the selective agent and streptomycin is ineffective under anaerobic conditions. Inclusion of vancomycin in blood agar was recommended by Finegold, Siewert, and Hewitt (Bacteriol. Proc., p. 59, 1957) for the isolation of fastidious anaerobes.

These media and various modifications of them were tested for the enumeration of leptotrichia and fusobacteria. It was found that Blood Agar Base (Difco), containing 5% defibrinated sheep

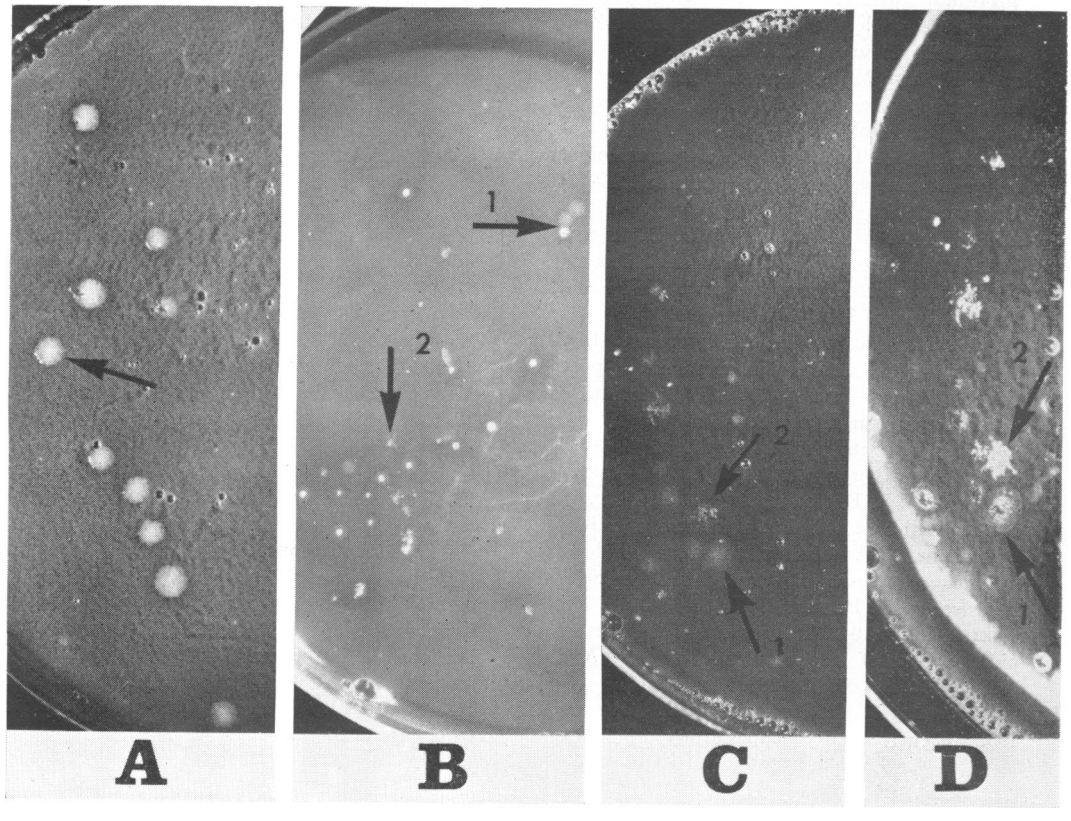


FIG. 1. *Selective medium plates inoculated with different oral plaque material. A, fusobacteria on chocolate agar after 4 days of incubation. B, blood agar after 3 days of incubation; 1, two types of fusobacterial colonies indicated; 2, a leptotrichia colony. C, chocolate agar after 3 days of incubation; 1, a fusobacteria colony; 2, a leptotrichia colony. D, same plate as C, but photographed with very oblique, reflected lighting.*

blood in the natural or the "chocolate" state with the addition of vancomycin (7.5 μg per ml of medium) and streptomycin (20 μg per ml), was an excellent medium for the isolation of these organisms.

As part of a project following the development of the oral flora in infants and children, plates of both types of blood agar containing the respective amounts of vancomycin and streptomycin have been included among the different media routinely inoculated with scrapings from teeth (oral plaque material). These and other plates to be cultured were incubated at 37 C for 3 to 4 days in an atmosphere of 90% hydrogen and 10% carbon dioxide.

The typical colonies obtained are shown in Fig. 1. By reflected light, leptotrichia appear as rough, whorled, colorless, flat, "Medusa head" colonies (B 2, C 2, and D 2). Fusobacteria form either raised, convex, glistening, speckled, cream-colored colonies (A) or colorless, shiny, flat, spreading colonies (B 1, C 1, and D 1).

We have cultured over 200 samples on this medium, which is highly selective for fusobacteria and leptotrichia; only occasionally were colonies of *Bacteroides* and *Neisseria* species observed.

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## REPLACEMENT OF NICOTINIC ACID REQUIREMENT OF *SHIGELLA SONNEI* BY PYRIDINE-3-SULFONIC ACID

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*Shigella* species require nicotinic acid for growth (Koser, Dorfman, and Saunders, Proc. Soc. Exptl. Biol. Med. **43**:391, 1940; Dorfman et al., J. Infect. Diseases **65**:163, 1939; Kligler and Grosowitz, J. Bacteriol. **38**:309, 1939; Weil and Black, Proc. Soc. Exptl. Biol. Med. **55**:24, 1944). Pan, Yee, and Gezon (J. Bacteriol. **83**:61, 1962) found that some, but not all, strains of *S. flexneri* required nicotinamide. We found that a number of compounds supported limited growth of *S. sonnei* when added to an otherwise complete medium, but that these were unable to sustain growth of subcultures in the absence of nicotinic acid. The compounds assayed were tryptophan, kynurenine, 3-hydroxykynurenine, kynurenic acid, hydroxyanthranilic acid, ornithine, proline, and hydroxyproline. Several of these substances have been reported to act as precursors of nicotinic acid (Leifer et al., J. Biol. Chem. **184**:589, 1940; Bovarnick, J. Biol. Chem. **151**:467, 1943; Beadle, Mitchell, and Nyc, Proc. Natl. Acad. Sci. U.S. **33**:155, 1947; Bonner and Yanofsky, Proc. Natl. Acad. Sci. U.S. **35**:576, 1949; Yanofsky and Bonner, J. Biol. Chem. **190**:211, 1951).

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Pyridine-3-sulfonic acid has been reported to inhibit the growth of a nicotinic acid-requiring strain of *Staphylococcus aureus*, presumably by acting as an antimetabolite (McIlwain, Brit. J. Exptl. Pathol. **21**:136, 1940). On the other hand, Lwoff and Querido (Compt. Rend. Soc. Biol. **130**:1569, 1939) reported that some organisms can use pyridine-3-sulfonic acid as a growth factor. We found that pyridine-3-sulfonic acid replaces the nicotinic acid requirement in *S. sonnei*, and that the organism may be maintained indefinitely upon subcultivation in a chemically defined medium (Erlandson and Mackey, J. Bacteriol. **75**:253, 1958) containing this substance as a substitute for niacin. A total of 13 strains of *S. sonnei* obtained from various sources were successfully maintained in culture media containing pyridine-3-sulfonic acid in place of niacin. The cultures were checked periodically for purity. The initial inocula were meticulously prepared by rinsing the cells in phosphate-buffered saline five times to minimize the possibility of nutrient carry-over. Each strain was studied in triplicate experiments. The growth of the organisms was measured turbidimetrically and by plate-counting methods.