Technical method

A semiselective medium for the isolation of *Veillonella* species from the mouth

T. WALLACE MACFARLANE *Microbiology Unit, Glasgow Dental Hospital and School, 378 Sauchiehall Street, Glasgow*

Veillonella species are commonly present in the human mouth and are strictly anaerobic Gram-negative cocci, 0.3-0.5 μm in diameter, usually arranged in pairs or masses. The generally accepted description of the oral species is that of Rogosa (1964; 1965). Although *Veillonella* species grow well on blood agar they are difficult to isolate from mixed specimens since they tend to be overgrown by streptococci and other oral bacteria. A selective medium for the isolation and enumeration of *Veillonella* species using basic fuchsin and streptomycin as inhibitory agents was described by Rogosa (1956). In a later paper, vancomycin was substituted for streptomycin (Rogosa *et al.*, 1958). The medium described in this article was developed as a result of an investigation to find a selective medium for the isolation of the Gram-negative members of the oral flora.

Material and methods

The composition of the basal medium was as follows: neutralised bacteriological peptone (Oxoid Ltd, Basingstoke, Hampshire) 24.4 g, purified agar (Oxoid) 14.6 g, L-cysteine hydrochloride (BDH Chemicals Ltd, Poole, Dorset) 0.6 g, yeast extract (Oxoid) 6.0 g, potassium nitrate (BDH) 1.2 g, and deionised water 1000 ml. The constituents were dissolved at 100°C and, when molten, the pH was adjusted to 7.4 and 25 ml of a 0.2% solution of bromothymol blue was added. The medium was dispensed in 100 ml amounts and autoclaved at 120°C for 15 minutes.

The inhibitory agent and various growth factors which were heat-sensitive were prepared separately as stock solutions in the following way: a 10% solution of glucose (BDH) in deionised water and a 70% w/w solution of sodium lactate (BDH) were sterilised by Tyndallisation. The vitamin K and haemin solutions were prepared as follows: solution

A—100 mg of menadione were added to 20 ml of 95% ethyl alcohol and sterilised by millipore filtration; solution B—50 mg of haemin (BDH) were dissolved in 1 ml of N NaOH, added to 100 ml of deionised water, and autoclaved at 115°C for 15 minutes. The stock solution of vitamin K and haemin was made by adding 1 ml of solution A to 100 ml of solution B. A 1% solution of Teepol 610 (Shell Chemical Company) was prepared in deionised water and autoclaved at 115°C for 15 minutes. To prepare the complete medium, 100 ml of the basal medium were melted and then cooled to 55°C when the following volumes of the stock solutions were added: sodium lactate 1.2 ml, glucose 10 ml, vitamin K/haemin 1.2 ml, and Teepol 610, 1.2 ml. The final mixture was dispensed in 15 ml amounts into sterile plastic Petri dishes, allowed to solidify, and dried at 50°C for 10 minutes before being used.

Two experiments were carried out to find the concentration of Teepol allowing maximum growth of *Veillonella* species and at the same time completely inhibiting the Gram-positive members of the oral flora. In the first experiment, plates were prepared with concentrations of Teepol ranging from 0.005 to 0.5% and inoculated with a standard loopful of emulsified dental plaque. The plates were placed in an anaerobic jar and inoculated for two days at 37°C. The Gas Pak system BBL (Baltimore Biological Laboratories: Division of Becton, Dickinson and Company, Maryland, USA) was used to obtain anaerobic conditions, and anaerobiosis was monitored by the use of methylene blue strips (BBL). Gram films were made from the resultant growth, and selected colonies were incubated aerobically and anaerobically on blood agar plates to assess their sensitivity to oxygen. Oxidase-negative, strictly anaerobic Gram-negative cocci were accepted as *Veillonella* species.

In the second experiment the growth of NCTC 9805, an anaerobic coccus of Hare's group V, was compared on media containing 0.01% and 0.1% Teepol. A smooth emulsion of an overnight blood agar culture of NCTC 9805 was made in 1 ml of sterile peptone water, and serial dilutions from 10^{-1} to 10^{-8} were prepared in the same medium. Using an Eppendorf pipette, 0.002 ml of each dilution was placed in the appropriate sector of the 0.01% and 0.1% Teepol plates. The plates were incubated anaerobically for two days. The number of resultant colonies were counted at the dilutions where this was possible. Using the same technique, the growth

of *Veillonella* species isolated from the human mouth was assessed on the 0.1% and 0.01% Teepol plates. A third experiment was carried out to assess whether the 0.01% Teepol medium had any inhibitory effect on *Veillonella* species. The techniques used were as previously described in the second experiment, except that growth on blood agar and 0.01% Teepol medium were compared.

Results

The results of the first experiment showed that the Gram-positive flora of the mouth was totally inhibited by 0.01% Teepol. On the other hand, 0.15% Teepol was necessary to inhibit the Gram-negative oral flora with the exception of *Veillonella* species, and a concentration of more than 0.5% Teepol was required completely to inhibit the growth of *Veillonella* species.

In the second series of experiments absence of growth occurred at a 10^{-4} dilution of NCTC 9805 and of *Veillonella* species isolated from the human mouth on the 0.1% Teepol medium, compared with a dilution of 10^{-8} on the 0.01% Teepol medium.

In the third series of experiments no significant difference was found between the growth of various dilutions of NCTC 9805 and of *Veillonella* species isolated from the human mouth cultured on blood agar and 0.01% Teepol agar. It can be concluded from these experiments that 0.01% Teepol agar has very little or no inhibitory effect on *Veillonella* species, while effectively inhibiting the oral Gram-positive flora.

On Teepol medium, *Veillonella* species produced green colonies 2-3 mm in diameter with raised centres and a rough or smooth edge after anaerobic incubation for one to two days. The colonial morphology of *Veillonella* species was strikingly different in size and colour when compared to the other Gram-negative bacteria which grew anaerobically on 0.01% Teepol plates. These organisms, which include *Haemophilus* spp., *Vibrio*, and *Fusobacteria*, produced small colonies 1 mm or less in diameter and were mainly clear or yellow in colour. In addition, many of these organisms required more than three days to produce macroscopic colonies, and if the plates were examined after 24-48 hours a 'pure'

culture of *Veillonella* species was often present.

Discussion

Teepol is a detergent used in many laboratories and pharmacies for cleaning glassware, and the active agent is based on the sodium and potassium salts of alkyl sulphates and alkyl aryl sulphonates. It is supplied in two forms, Teepol L and Teepol 610. Since Teepol 610 has a standard composition whereas the composition of Teepol L varies with different batches, the former reagent should be used for media preparation. Teepol 610 has been used in place of bile salts as a selective agent in the bacteriological examination of water (Jameson and Emberley, 1956) but the inhibitory effect on the oral microflora has not previously been described.

These experiments show that Teepol medium (0.01%) is non-inhibitory to *Veillonella* species when compared to blood agar. The Teepol medium is primarily intended for routine isolation of *Veillonella* species, and in this situation surface plating is more suitable than the pour-plate technique which is recommended when using Rogosa's medium.

No difficulty was experienced in the isolation of *Veillonella* species from saliva, tongue scrapings, throat swabs, and dental plaque. The Teepol medium can be used as an alternative to Rogosa's medium for the isolation of *Veillonella* species, and it has the added advantage of inhibiting the oral Gram-positive flora while having little effect on the Gram-negative flora.

References

- Jameson, J. E. and Emberley, N. W. (1956). A substitute for bile salts in culture media. *Journal of General Microbiology*, **15**, 198-204.
- Rogosa, M. (1956). A selective medium for the isolation and enumeration of the *Veillonella* from the oral cavity. *Journal of Bacteriology*, **72**, 533-536.
- Rogosa, M. (1964). The genus *Veillonella*. 1. General cultural, ecological, and biochemical considerations. *Journal of Bacteriology*, **87**, 162-170.
- Rogosa, M. (1965). The genus *Veillonella*. 4. Serological groupings and genus and species emendations. *Journal of Bacteriology*, **90**, 704-709.
- Rogosa, M., Fitzgerald, R. J., MacKintosh, M. E., and Beaman, A. J. (1958). Improved medium for selective isolation of *Veillonella*. *Journal of Bacteriology*, **76**, 455-456.