HYDROGEN SULPHIDE PRODUCTION AS AN AID TO THE IDENTIFICATION OF *VIBRIO PARAHAEMOLYTICUS*

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INTRODUCTION

Vibrio parahaemolyticus is being isolated from specimens of diarrhoea cases with increasing frequency in Malaysia in recent times (Puthucheary, 1973; Jegathesan and Paramasivam, 1976). It has also been isolated from a high proportion of Malaysian shellfish examined for its presence (Jegathesan *et al.*, 1976). The current increased isolation rates are probably due to the fact that we have only recently begun to look seriously for the organism.

This paper describes that *Vibrio parahaemolyticus* strains are seen to produce H_2S when inoculated into Russel's Triple Sugar slopes. This phenomenon has a characteristic appearance and allows an unknown strain to be suspected to be *Vibrio parahaemolyticus.*

MATERIALS AND METHODS

In this study 18 strains isolated from shellfish and 8 strains from humans were confirmed to be *Vibrio parahaemolyticus* by the following criteria:

Morphological appearance on Monsur's agar and TCBS., Growth in 1%, 3% and 7% NaCl but not in 10% NaCl; positive oxidase, catalase, lysine and ornithine decarboxylase, indole and methyl red tests; negative arginine decarboxylase test; fermentation of glucose and mannitol; no fermentation of sucrose; agglutination with pooled K *Vibrio parahaemolyticus* antiserum.

All strains were then inoculated into slopes of Russel's Triple Sugar (RTS) by first smearing the slopes and then stabbing the butt. These were then incubated at 37°C and observed at 24 and 48 hours. The RTS was made up as follows:

To 200 ml of melted stock agar (made up of 0.4% NaCl, 0.6% "oxoid" Lab. lemco, 4% "Bacto" peptone, 3.5% "Bacto" agar) is added 200 ml of 0.1% lead acetate solution, 4 gm of lactose, 4 gm of sucrose, 0.2 gm of glucose and 12 ml of 3% Andrade's indicator are then added.

Representative strains were also inoculated onto TSI slopes and RTS slopes made up as above except that phenol red was substituted for Andrade's indicator. A set of inoculated RTS slopes were also incubated at room temperature and under anerobic conditions. A set of RTS slopes without lead acetate was also inoculated. Representative strains of *Salmonella*, *Shigella*, *E. coli* and *Proteus* were also inoculated on to RTS as controls.

RESULTS

On RTS slopes with Andrade's indicator, all the strains of *Vibrio parahaemolyticus* tested showed a characteristic surface browning of the slope which was faintly visible after overnight incubation but accentuated after 48 hours. The browning started at the very tip of the slope and gradually spreaded downwards. Control *Salmonella* and *Proteus* organisms gave browning only in the region of the stab and not on the surface of the slope. This browning phenomenon was not seen in TSI, on RTS with phenol red as indicator and on RTS incubated anaerobically. RTS incubated at room temperature showed a slightly delayed reaction.

The phenomenon was not seen on RTS slopes without lead acetate showing that it is due to H_2S production.

DISCUSSION

The results of the above study indicate that strains of *Vibrio parahaemolyticus* can be shown to produce hydrogen sulphide in a characteristic manner when grown on RTS slopes.

Most of the literature reviewed described *Vibrio parahaemolyticus* as being a non-producer of hydrogen sulphide (Sakazaki *et al.*, 1963; Fujino *et al.*, 1972; Puthucheary, 1973; Zen Yogi *et al.*, 1973). However, Twedt *et al.*, (1966) showed that H_2S production from *Vibrio parahaemolyticus* was seen in 56 of 57 instances when SIM medium was used and in 48 out of 57 cultures when lead acetate agar (Difco) was used. No H_2S production was visible on TSI. The medium employed has a profound effect on the test results. H_2S production is widespread among organisms and is dependant on their ability to decompose sulphur containing amino acids. It is relative and depends on the different sensitivities of different media. For purposes of using H_2S production as a differentiating test for organisms it is therefore important that a definite level of sensitivity of the test media should be maintained. The indicator used also appears to have an effect on the test results. Andrade's indicator which gives only a light colouring to the medium allows the browning to be visible while the darker phenol red masks it.

The surface browning seen in our experience could be due to the fact that *Vibrio parahaemolyticus* strains are more capable of producing H_2S under aerobic conditions because of enhanced growth. There was no or very slight growth under anaerobic conditions and no H_2S was seen to be produced.

The value of the observation that *Vibrio parahaemolyticus* produces H_2S in this fashion is that it allows one to suspect that an organism causing this effect on RTS could in fact be *Vibrio parahaemolyticus* and the relevant biochemical and agglutination tests can then be performed to confirm this suspicion.

SUMMARY

In this study 18 strains of *Vibrio parahaemolyticus* from food and 8 from humans were tested for hydrogen sulphide production on various modifications of Russel's Triple Sugar slopes and on TSI. All strains showed a characteristic surface browning on RTS with Andrade's indicator. This was not seen when RTS with phenol red as indicator or TSI were used. Appearance of this phenomenon allows unknown strains to be suspected as being *Vibrio parahaemolyticus*.

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