SULFUR BACTERIA FROM A SOUTHWESTERN FLORIDA SINKHOLE: A PRELIMINARY REPORT

J. H. Tuttle and S. J. Molyneaux

Woods Hole Oceanographic Institution

Woods Hole, Massachusetts 02543

INTRODUCTION

The recent discovery of a sulfide-containing sinkhole located near Venice, Florida has prompted interest among biologists, geologists, and archaeologists. The sink, about 220 feet deep (67 m), has been found to contain human bones, well preserved by hydrogen sulfide in the water which fills the hole. The saline water has a chlorinity of about 9.05 $\pm$ 2‰ (F. A. Kohout, U.S. Geological Survey). Warm water (ca. 38C) enters at the bottom of the sink at a rate of about 8.6×10^5 ft^3 per day. Considering the volume of the hole, the water has a residence time of about 20 days.

Our interest in the sinkhole stems from ongoing research concerning the microbial sulfur cycle in anoxic marine environments. In this report, we present some preliminary information on sulfur bacteria which we have cultured from a series of samples collected from the sink during the spring of 1975.

MATERIALS AND METHODS

Sampling

Samples were taken by SCUBA divers using sterile 50 ml plastic syringes. Immediately upon reaching the surface, the syringe contents were transferred into sterile, 35 ml glass bottles which had been pre-gassed with O_2-free N_2 gas and fitted with butyl rubber stoppers. The bottles were filled to overflowing with the water sample and immediately re-stoppered.

Enrichment

Media and culture conditions. Media for *Thiorhodaceae* were prepared according to Postgate (1967). Specific enrichment cultures were made for the following groups: *Chromatium* sp. and *Thiopedia; Chlorobium* sp.; and *Chlorobium thiosulfatophilum*. The culture medium with a salinity of 18% were added to sterile, gassed out 35 ml bottles and inoculated with 5 ml of sample. The bottles were incubated at 1 to 2 feet from a 40 watt incandescent light for a period of 3 months at 25 ± 3C.

Enrichment cultures for chemolithotrophic sulfur bacteria were made in the thiosulfate medium (TB) of Tuttle and Jannasch (1972) at 18% salinity and in sulfide gradient cultures containing 10 ml of a 4% NaCl, 4% agar solution overlayed after solidification with about 70 ml of the TB medium at pH 7. The TB medium cultures, contained in 16 x 120 mm test tubes, consisted of 5 ml of medium at pH 7 and a 1 ml inoculum; and the gradient cultures were prepared in 100 ml culture tubes with an inoculum of 5 ml. The cultures were incubated stationary at 25 ± 3C.

Isolation procedure. Isolations were made from the TB medium and sulfide gradient cultures by streaking onto TB agar plates as described previously (Tuttle and Jannasch, 1972). Isolated colonies were subcultured and maintained on TB agar slants. The isolates were compared on the basis of their colony morphology on TB agar and Nutrient Agar (Difco) containing 2% NaCl. Duplicate isolates were discarded.

Physiological testing. The representative isolates were tested for their ability to oxidize thiosulfate under aerobic conditions and to reduce

tetrathionate and thiosulfate under anaerobic conditions. Thiosulfate oxidation was determined in TB medium as previously described (Tuttle and Jannasch, 1972). Following incubation for 14 days at 22 ± 2C, the culture fluids were analyzed for pH, thiosulfate, tetrathionate, trithionate, and total cell protein. Uninoculated medium served as the control.

Determinations of anaerobic growth and tetrathionate or thiosulfate reduction were made using the pyruvate medium of Tuttle and Jannasch (1973). Following incubation at 22 ± 2C as noted in Tables 2 and 3, the cultures were examined for pH, thiosulfate, tetrathionate, trithionate, sulfide, and cell protein. Controls consisted of uninoculated medium with or without tetrathionate or thiosulfate.

Chemical determinations. Sulfide in the original samples was measured by the method of Packmeyer (quoted by Trüper and Schlegel,1974) and in the anaerobic growth experiments by the technique of Gilboa-Garber (1972). The latter method is not affected by the presence of thiosulfate at high concentration (Tuttle and Jannasch, 1973).

Thiosulfate, tetrathionate and trithionate were measured using the cyanolysis method of Kelly *et al.* (1969). Where necessary, sulfide interference was removed using the modification described by Tuttle and Jannasch (1973).

Total cell protein was determinated by the procedure of Lowry *et al.*; (1951) after the cell material had been worked with artificial seawater and digested with 10% (w/v) trichloracetic acid. The pH was measured with a Metrohm expanded scale pH meter (Brinkman Instruments).