Biphasic Thiosulphate Utilization by a Marine *Thiobacillus*

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Most reports concerning the oxidation of reduced sulphur compounds have involved the chemolithotrophic micro-organisms of the genus *Thiobacillus*. Three of these reports (Tilton, Cobet & Jones, 1967*a*; Tilton, 1968; Adair & Gunderson, 1969*a*) were concerned with the distribution, and a fourth (Tilton, Stewart & Jones, 1967*b*) with fine structure of organisms isolated from ocean waters. Adair & Gunderson (1969*b*) characterized a facultative autotrophic *Thiobacillus* isolated from the Pacific Ocean. In this communication, the biphasic utilization of thiosulphate by the marine isolate, *Thiobacillus* sp. IV-85 is described.

METHODS

A culture of an unknown *Thiobacillus*, designated *Thiobacillus* sp. IV-85, was isolated from the Atlantic Ocean according to the procedures of Tilton (1968). The organism was maintained on thiosulphate salts broth of the following composition: 24.81 g. $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$; 2.20 g. NH_4Cl ; 2.00 g. KH_2PO_4 ; 500 ml. synthetic sea water (Lyman & Fleming, 1940); and 500 ml. distilled deionized water. The pH of the medium was adjusted to 5.0 with 5*N*-HCl and the medium was steam sterilized at 121° for 15 min. Bacteria were grown in 1.0 l. cultures at 20°, shaken at 200 rev./min. Thiosulphate was determined by iodine titration using a starch indicator. Total polythionates were determined according to the method of Starkey (1934). Triplicate plating of suspensions on thiosulphate salts agar gave wide variation in viable count; therefore, total count was determined with a Petroff–Hausser counting chamber.

RESULTS AND DISCUSSION

Adair & Gunderson (1969*a*) reported that at least 50 % sea water was required in their thiosulphate salts broth for the optimal growth of two thiobacilli isolated from the Pacific Ocean. Fig. 1 shows that the marine isolate *Thiobacillus* sp. IV-85 grew equally well whether the growth medium was supplemented with or devoid of synthetic sea water. The length of the lag periods in those flasks containing 100, 80, 60 and 40 % synthetic sea water were essentially the same. The length of the lag period in those flasks containing 20 % synthetic sea water was slightly longer, whereas the lag period in those flasks devoid of sea water was greatly extended. Fig. 1 also shows that in all cases, irrespective of the concentration of sea water, thiosulphate utilization during the growth of *Thiobacillus* sp. IV-85 proceeded in two sequential logarithmic phases. Biphasic utilization of thiosulphate by a *Thiobacillus* has not hitherto been reported in the literature. A full log plot of the total count against thiosulphate utilization showed a direct correlation between the disappearance of thiosulphate and growth of the organism.

In 50 % synthetic sea water+thiosulphate salts medium the first logarithmic phase of growth was characterized by an increase in the pH from 5.0 to 5.75. The concentration of

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polythionates also increased during the first log phase. The increase in pH reached a maximum at the time of the transition from the first to the second log phase of growth. During the second logarithmic phase the pH decreased, reaching 4.7 at the time of complete thiosulphate disappearance. There was then a rapid decrease in the concentration of polythionates and a concomitant decrease in the pH. The final pH after 72 h. was 2.4. Generation times for the first and second logarithmic phases were 2.8 and 7.6 h. respectively.

Fig. 1. Thiosulphate utilization during growth of *Thiobacillus* sp. 1V-85 in thiosulphate salts medium supplemented with varying concentrations of sea water: 100 %, ●; 80 %, ○; 60 %, ▲; 40 %, △; 20 %, ■; 0 %, □.

Little is known of the role that marine thiobacilli play in the biogeochemistry of sulphur in the marine environment, and less is known of the physiology of these thiobacilli. Tilton (1967*a*, 1968) and Adair & Gunderson (1969*b*) reported that their isolates were marine counterparts to thiobacilli isolated from terrestrial sources. Results from our laboratory indicate that all terrestrial strains so far examined utilize thiosulphate in a single logarithmic rate during 'log phase' growth.

Since the oxidation of thiosulphate provides the energy and reducing power for growth of the thiobacilli, the abrupt alteration in the rate of thiosulphate oxidation by *Thiobacillus* sp. 1V-85 suggests that one (or more) of the mechanisms of thiosulphate oxidation is inhibited.

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