Isolation and Cultivation of Iron And Sulfur Bacteria from Domestic Sewage

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INTRODUCTION

For the last hundred years it has been known that corrosion and tubercle formation in piping is often caused or aggravated by an iron-consuming or iron-depositing bacteria, such as *Gallionella* or *Clonothrix*. In 1870 Cohn presented a theory to explain the bacterial precipitation of iron, and in 1949 Winogradsky explained the physiology of the nitrifying and sulfur bacteria. Iron and sulfur bacteria play a major economic role in the oil industry and in municipal sewage and water systems. Sulfate reducing bacteria are capable of forming large quantities of hydrogen sulfide from the sulfates that are present in most injection waters. This hydrogen sulfide in turn reacts with any iron present in the water to form insoluble iron sulfide; the result is a rapid plugging of sewer pipes, or in the case of the oil industry, plugging of water injection wells.

Brown (1934) isolated iron bacteria from water supplies, and R. S. Wolfe in 1957-1960 classified iron bacteria from samples taken from the Richmond Water Corporation. Pringsheim (1949) has shown *Sphaerotilus* sp. to be associated with polluted water.

We isolated iron and sulfur bacteria from the Norman, Oklahoma sewage influent and, using morphological and growth characteristics, identified them as, or similar to, *Clonothrix putealis, Gallionella ferruginea* and *Sporovibrio desulfuricans*. These organisms, so far as known, have not been isolated previously from domestic sewage.

MATERIALS AND METHODS FOR ISOLATION OF IRON BACTERIA

A 200 ml sample of sewage was collected from the side of the grit chamber at the Norman sewage treatment plant and was used as an inoculum . The source of iron (ferrous sulfide) for the cultivation of iron bacteria was prepared as described by Kucera and Wolfe (1957). The sterilized ferrous sulfide was mixed with an equal volume of sterile melted agar at 45 C. The mixture was slanted in screw-cap tubes and a liquid medium of NH_4Cl 1g/l., $K_2HPO_4$0.5g/l.,$MgSO_4$ 0.2g/l., and $CaCl_2$ 0.1g/l was then added. CO_2 was bubbled through this medium for 10-15 seconds before it was added to the test tubes. The tubes were then inoculated with a loopful of sewage and incubated at room temperature for 24-48 hours.

The above mentioned method was also duplicated with the difference that instead of screw-cap tubes, Coplin jars were used. The sterilized media was poured into Coplin jars, and microscopic slides were then set in. The jars were incubated thereafter under the same conditions as were the tubes in the original procedure.

RESULTS

Macroscopically the colonies appeared white and cottony after 18-24 hours and gradually turned a rust color on aging. Macroscopic examination of the colonies developed on the slides was made after fixing and staining in prussian blue. A drop of 0.2M HCl was added to the air-dried colonies on the slide followed by 1% $K_4Fe(Cn)_6$. A deep blue color resulted. The slides were washed, air dried, and then counterstained in crystal violet.

The slides from the Coplin jars were a great improvement over the slides made by looping samples out of the tubes. Not only was it much

easier to handle the specimens, but the growth characteristics of the bacteria were shown to better advantage. The organisms and their stalks were usually deteriorated and broken in the case of the slides made from the tube cultures. Morphologically, twisted stalks and filamentous organisms were observed. They appeared to be *Gallionella* sp., *Crenothrix* sp., and *Clonothrix*. Examples are shown in Fig. 1(a), 1(b), and 1(c).

Materials and Methods for Isolation of Sulfur Bacteria

The medium used is similar to that of Allred (1958).

Sodium lactate	4.0	ml
Yeast extract	1.0	g
Ascorbic acid	0.1	g
$MgSO_4 \cdot 7H_2O$	0.2	g
K_2HPO_4 (anhyd)	0.01	g
NaCl	10.0	g
$Fe(SO)_4(NH_4)_2 \cdot 6H_2O$	0.1	g
Agar	15.0	g
Distilled water	1000.0	ml

The ingredients were dissolved with gentle heating and pH adjusted to 7.5 with NaOH. The medium was autoclaved for 10 minutes at 15 lbs. pressure and poured into screw cap tubes after cooling to 45 C. Five tubes were stabbed with a loopful of sewage suspension and incubated at room temperature under anaerobic conditions in a Brewer jar for a week.

Results

Macroscopically the colonies appeared brown to golden color along the stab, and the surrounding media was black indicating release of hydrogen sulfide by the organisms. Microscopic examination of stained hanging drop preparations showed spirally, motile, gram negative vibrios. *Sporovibrio desulfuricans,* shown in Fig. 2.

Discussion and Summary

Samples of sewage were taken from the Norman, Oklahoma sewage treatment plant. The enrichment medium used for the isolation of iron bacteria was that of Kucera and Wolfe (1957). Although much work has been done on the isolation of iron bacteria, particularly *Gallionella, Crenothrix,* and *Lepthothrix,* there is no report to our knowledge of their isolation from domestic sewage.

The method we used to cultivate iron bacteria is believed to be a substantial improvement over the usual tube method. The slides in the Coplin jars permitted the growth and subsequent preparation of slides exhibiting oganisms with undistorted growth patterns. The iron deposition as $Fe(OH)_3$ in the sheath of the organisms was confirmed by the prussian blue reaction, which also facilitated examination. The organisms observed are most probably *Gallionella ferruginea,* and *Clonothrix putealis.* All attempts to get a pure culture of iron bacteria failed. Sulfur bacteria *(Sporovibrio desulfuricans)* were isolated by using the enriched medium of Allred (1958).

Acknowledgements

We wish to thank Dr. Robert E. Collier, Dr. Norman H. Boke, and Dr. R. S. Wolfe. Dr. Collier helped to identify the organisms; Dr. Boke assisted in taking the photomicrographs; and Dr. Wofe contributed many valuable suggestions.

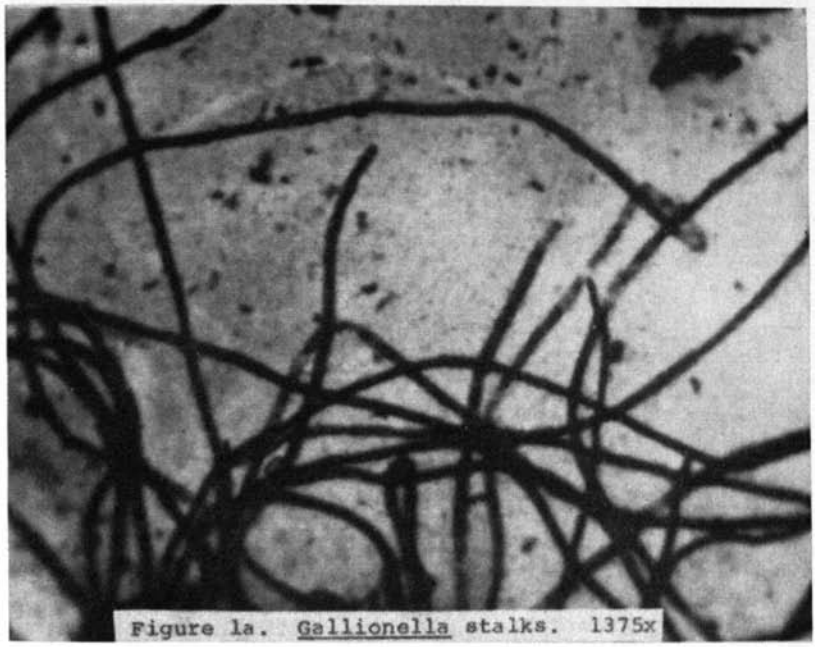

Figure 1a. Gallionella stalks. 1375x

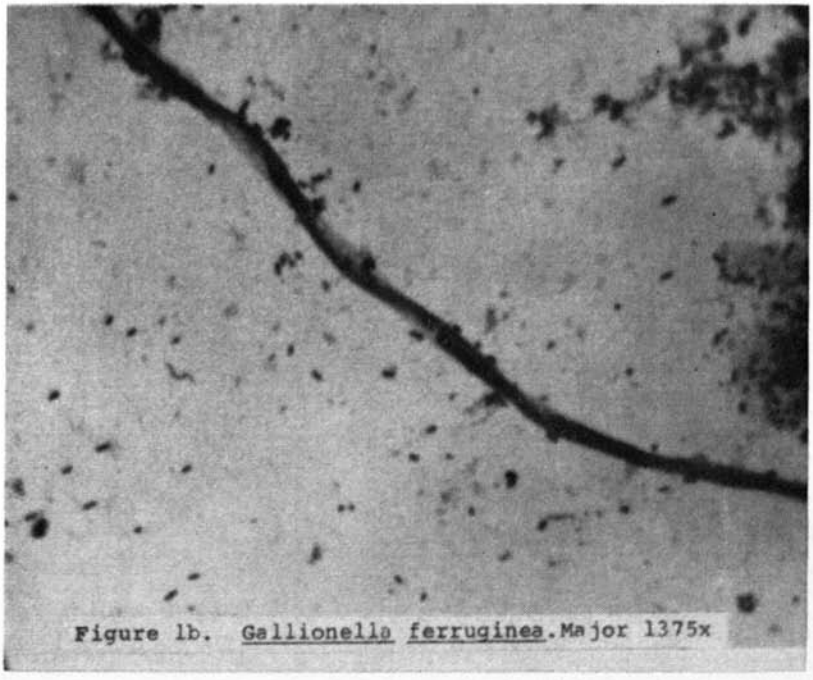

Figure 1b. Gallionella ferruginea. Major 1375x

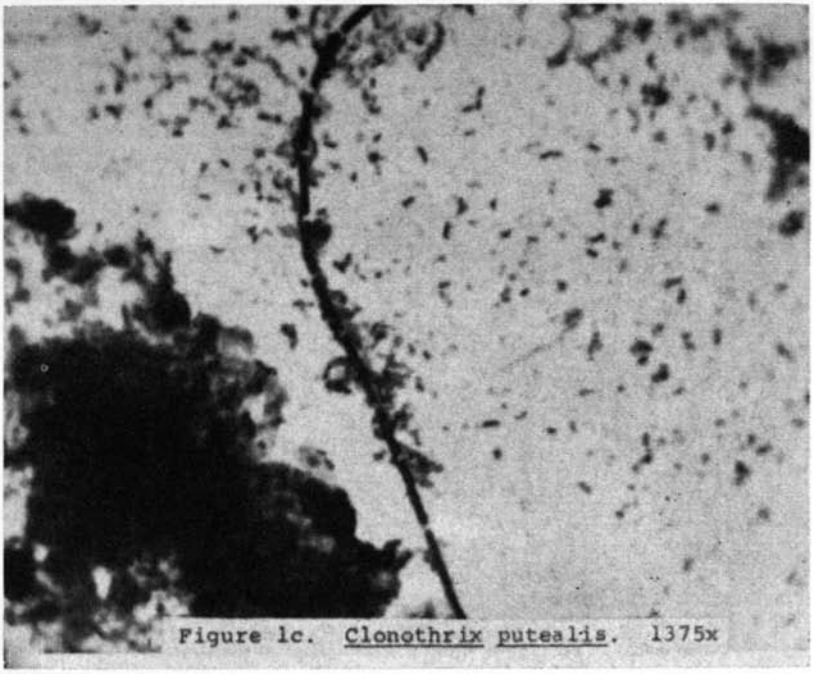

Figure 1c. Clonothrix putealis. 1375x

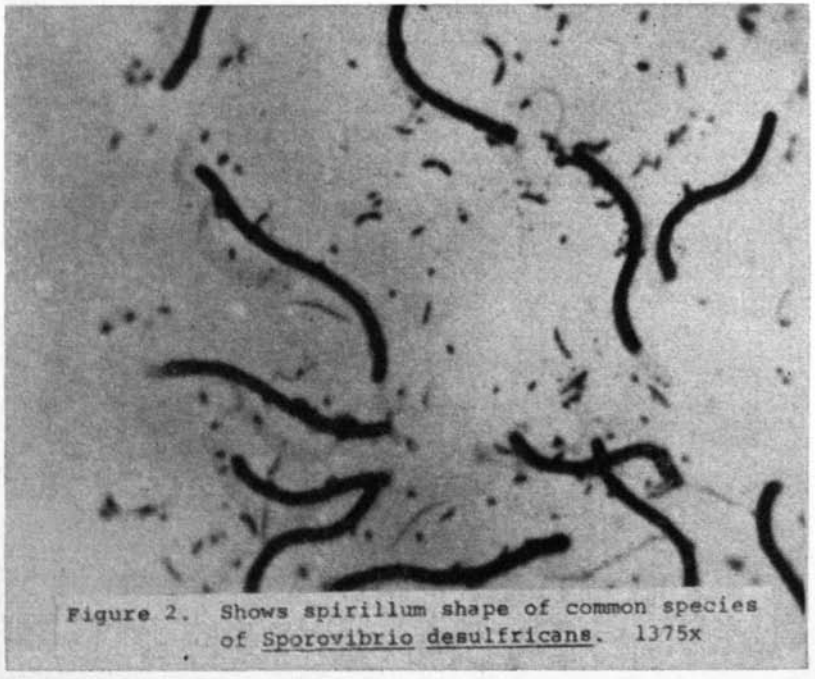

Figure 2. Shows spirillum shape of common species of Sporovibrio desulfricans. 1375x

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