STUDIES ON MICROBIOLOGICAL CORROSION OF METALS: PART I— ISOLATION AND IDENTIFICATION OF SULPHATE REDUCING BACTERIA FROM JODHPUR SOIL

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A strain of sulphate reducing bacteria present in Jodhpur soil has been isolated and identified as *Desulphovibrio desulphuricans*.

During the course of our studies on underground corrosion of metals in Jodhpur, the formation of black corrosion products in the case of cast iron was observed ; also the presence of ferrous sulphide in addition to ferric oxide was detected in the corrosion product. This suggested the possible presence of sulphate reducing bacteria in the soil. Therefore detailed studies were undertaken to isolate and identify the bacteria present in Jodhpur soil.

MATERIALS AND METHOD

Soil samples were collected from depths of 2, 4, 6, and 8 feet. The *pH* of the soil, its water soluble contents and H_2S production due to the presence of sulphate reducing bacteria, are given in Table 1. About 0·5 to 1·0 *g* of the sample was taken in a 60 ml glass stoppered bottle[1] and filled with Starkey's medium[2] ensuring that no air bubbles were entrapped. The system was incubated at various temperatures between 28° and 55°C and it was found that blackening of the medium was due to sulphate reduction by the bacteria occurred within 10 days in the case of samples incubated at 30°C. The H_2S production was observed only in the case of the three samples collected from the depths of 4, 6, and 8 feet and was in the range of 200 ppm indicating the presence of sulphate reducing bacteria[1].

The above bacterial culture was transferred to a fresh medium containing 3% of sodium sulphite ($Na_2SO_3 . 7H_2O$), which has been reported to inhibit the growth of obligate non-halophilic sulphate reducers[3], and incubated at 30°C. The reculturing in sulphite medium was repeated six times so as to obtain a crude culture free from most of the contaminating organisms. This was again recultured repeatedly in solid agar medium in plates and tubes[4] and incubated at 30°C in McIntosh and Fildes anaerobic jars filled with nitrogen. The final strain, thus obtained, was subjected to various tests for identification of the same[5].

RESULTS

The bacteria were found to grow under strictly anaerobic condition at an optimum temperature of 30°C. The bacteria did not grow at 55°C. It was found to be gram-negative, non-sporulating and mesophilic type. When viewed under the electron

TABLE 1

CHARACTERISTICS OF JODHPUR SOIL AT DIFFERENT DEPTHS

Characteristics	Soil sample collected from depth of			
	2 ft	4 ft	6ft	8 ft
pH of soil extracts (1:2·5)	8·06	8·00	8·12	8·14
Water soluble salts :				
(*i*) Anions (%)				
CO_3—	Nil	Nil	Nil	Nil
HCO_3—	0·015	0·015	0·045	0·012
Cl—	0·005	0·080	0·005	0·004
SO_4—	0·011	0·002	0·0015	0·001
NO_2—	Nil	Nil	Nil	Nil
NO_3—	0·0012	0·0006	0·0010	Nil
(*ii*) Cations (%)				
Ca—	0·0010	0·0018	0·0004	0·0006
Mg.—	0·0018	0·0091	0·0020	0·0031
Na—	0·0050	0·0048	0·0040	0·0055
K—	0·0008	0·0006	0·0005	Traces
Blackening of medium occurs in (hr)	No blackening	140	120	96
H_2S produced in 21 days (ppm)	—	229	225	229

microscope[6] the cells appeared to be slightly curved with occasional straight cells (see Fig. 1). Also mostly one polar flagellum was observed. These show that the bacteria were vibrio and monotrichous.

The influence of various components of the media on the growth of the bacteria indicated by the blackening of the medium and the quantities of H_2S produced (wherever considered essential) are given in Table 2. It is seen from Table 2 that the bacteria were able to grow in the presence or absence of peptone or yeast in the medium and were able to utilise thiosulphate or sulphite in place of sulphate. It was able to tolerate salt to the extent of 2·5% and grow in the presence or absence of salt in the medium. Alcohol was found to stimulate their growth. Use of sodium oxalate, sodium nitrate, sodium nitrite or glucose in place of sodium lactate in the medium inhibited the growth of the bacteria whereas sodium formate supported growth. They were able to grow in presence of sodium acetate, sodium butyrate or sodium

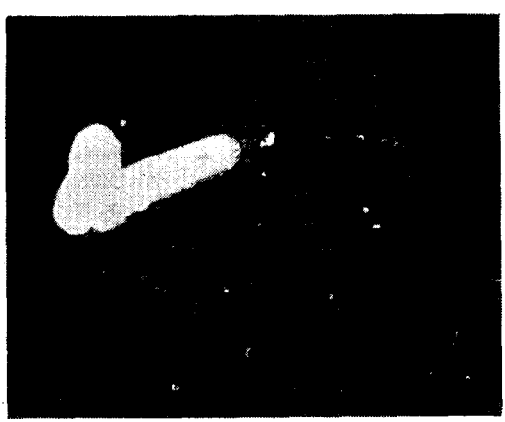

Fig. 1—*Desulphovibrio desulphuricans* cells of a strain from Jodhpur soil cultured in Starkey's medium 16, 000X

TABLE 2

EFFECT OF ALTERING THE COMPOSITION OF STARKEY'S MEDIUM ON THE GROWTH OF SULPHATE REDUCING BACTERIA

Details	Blackening occurs in (hr)	H_2S (ppm)	Hydrogen absorption coefficient (mm^3/mgcell/hr) $-QH_2$
Starkey's medium			
(*i*) + peptone	72—96	96	95
(*ii*) + yeast ext.	48—72	162	161
(*iii*) + without yeast	96—120	98	98
Starkey's medium without sulphate			
(*i*) + Sod. thiosulphate	72	—	—
(*ii*) + Sod. sulphite	96	—	—
Starkey's medium			
(*i*) + 1·5% NaCl	120	—	—
(*ii*) + 2·0% NaCl	120	—	—
(*iii*) + 2·5% NaCl	120	—	—
(*iv*) + 3·0% NaCl	No blackening	—	—
Starkey's medium + alcohol	48	—	—
Starkey's medium excluding Sod. lactate			
(*i*) + Sod. oxalate	No blackening	—	—
(*ii*) + Sod. nitrate	do	—	—
(*iii*) + Sod. nitrite	do	—	—
(*iv*) + Sod. formate	72	—	—
(*v*) + Glucose	No blackening	—	—
(*vi*) + Sod. acetate			
(1) with yeast	72	29	—
(2) without yeast	No blackening	—	—
(*vii*) + Sod. butyrate			
(1) + yeast ext.	72	13	—
(2) without yeast ext.	No blackening	—	—
(*viii*) + Sod. propionate			
(1) + yeast ext.	72	—	—
(2) without yeast ext.	No blackening	—	—
(*ix*) + Alcohol	72	—	—

propionate in place of sodium lactate in the medium provided yeast was present. This last characteristic indicated that the bacteria did not belong to the species *Desulphovibrio rebentschikii*[7].

The bacteria were not able to liquefy gelatin indicating the absence of proteolytic activity. Experiments carried out with Warburg's apparatus showed the presence of hydrogenase activity. The values of the hydrogen absorption coefficient are given in Table 2. Redox measurements in bacterial culture[9] showed that methylene blue (0·5 %

was decolourised within 18—24 hours (E_h — 0·24 volt) during their growth. These characteristics distinguished them from *D. orientis* type. The most probable number of bacteria[10] present in a 10-day old culture was found to be of the order of 10^5 per ml.

CONCLUSION

It is concluded from this study that the type of sulphate reducing bacteria present in Jodhpur soil is *Desulphovibrio desulphuricans*.

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