A PRELIMINARY STUDY OF THE BACTERIAL FLORA ASSOCIATED WITH SULPHUR DEPOSITS ON THE EAST COAST (MASULIPATAM)

THE soil collected from the Masulipatam area was first passed through Molisch's Enrichment Medium composed of Peptone 5 gms., Dextrin or Glycerin 5 gms., Seawater 1,000 ml., and Agar 18 gms., and with a pH of 7·6. The slants were inoculated with the samples of soil and incubated at 30° C. for 72 hours. Profuse growth was observed after 24 hours. This culture was then transferred to McGreger and Skene's medium (composition: ammonium sulphate 0·75 gm., magnesium sulphate 0·05 gm., potassium dihydrogen phosphate 0·05 gm., potassium chloride 0·05 gm., calcium nitrate 0·01 gm., sodium chloride 27·0 gms., calcium carbonate 10·0 gms., and distilled water 1,000 ml., pH 7·6). This is a synthetic medium which is expected to promote the growth of only the obligate or facultive autotrophs. The medium (50 ml.) was placed in 150 ml. Erlenmeyer flasks, sterilised and incubated at 30° for 15 days. The growth on this medium was poor and some flasks showed no growth at all.

Plating trials in McGregor and Skene's agar medium followed by 48 hours' incubation at 30° yielded distinct colonies. They were transferred to McGregor and Skene's agar slants. Three such passages through a completely synthetic medium were expected to eliminate all saprophytic contaminants. The organisms thus isolated appeared to constitute a pure culture as judged by microscopic characters and reaction to staining.

From a total of five samples of soil 14 distinctive stock cultures were obtained. They were subjected to the following studies:—

Morphological: (*a*) *Microscopic observation.*—The culture was first passed through a liquid medium of McGregor and Skene's composition and 24-48 hour culture studied for motility and Gram staining. (*b*) *Growth.*—Growth on nutrient agar, wort agar, McGregor and Skene's agar and Van Delden's sodium lactate-asparagin agar were studied.

Biological.—Since the temperature of incubation and pH of media were maintained as nearly the same as occurred in soil, no special trials were made in this connection. Observations were, however, made in all solid cultures as to their ærophilic, micro-ærophilic or anærophilic nature.

Biochemical: (*a*) *Tolerance of H_2S.*—Two sets of Erlenmeyer flasks with necessary controls containing McGregor and Skene's medium were inoculated with the cultures. To one set 5 c.c. of a saturated solution H_2S in water was added on alternate days, and the other set incubated without any such addition. Most of the cultures survived the first two doses only. (*b*) *Nitrate reduction.*—The cultures were inoculated into a medium to test for their nitrate-reducing properties. After 48 hours' growth the reduction was tested by Glossway's method. A control for nitrate and one for nitrite was also run. (*c*) *H_2S production.*—The organisms were inoculated into nutrient agar to which 0·1 per cent. of a 10 per cent. lead acetate solution had been added. None of the cultures showed any production of H_2S.

Thiosulphate reduction.—Waksman's thiosulphate medium composed of sodium thiosulphate 5 gms., potassium dihydrogen phosphate 3 gms., ammonium chloride 0·1 gm., magnesium chloride 0·1 gm., calcium chloride 0·25 gm., and distilled water 1,000 ml., was inoculated and incubated for 72 hours. An aliquot was titrated against standard iodine solution using starch as an indicator. The uninoculated flasks served as the control. None of the cultures had utilised any thiosulphate.

In interpreting the data given in Table I the following observations are relevant: (1) The area from which the soil was collected is washed by the backwaters of the sea and is submerged under stagnating water during the monsoon. (2) During the dry season the soil cracks and deep fissures are created. (3) At a depth of 3 feet or more the soil strongly smells of sulphuretted hydrogen. (4) The soil is associated with layers of red ochre consisting largely of ferric oxide. (5) In places where watersprings can be dug out a vigorous evolution of gas (later identified as marsh gas) is observed. The most significant circumstance was the occurrence of H_2S in the deeper strata of the soil and it was thought that some of the organisms might require its presence for their metabolism.

Sulphates constitute an important source of H_2S. Since the transformation of sulphate to H_2S is uneconomical to microbial life in that this change does not give them any energy surplus, the phenomenon is not common. However Beijerinck[1] reported Spirillum desulpuricans and Vandelden[2] reported microspira æstuarii. The oxygen liberated during the reduction of sulphate by these organisms is used up in oxidising some organic matter as was shown by Van Delden who used sodium lactate for the purpose. At room temperature the reduction was brought about in 5-10 days. Elion[3] has reported another organism Vibrio thermodesulphuricans able to bring about the reduction in 12 hours incubated at 50° C. The production of H_2S from organic matter is a phenomenon of more frequent occurrence and a large number of Saprophytes are known to achieve this. The absence of any production

Culture No.	* Microscopic characters and gram staining	† Growth on media	§ Nitrate reduction
1	Thin long rods; some sporulated; motile; −ve	N + −; W + + −; M.S. + + + +; V.D. + + + +	+ +
2	Short rods; nonmotile; −ve	N + +; W + + −; M.S. + + −; V.D. + + −	+
3	Long spirally coiled rods; nonmotile; −ve	N +; W + +; M.S. + +; V.D. + +	+ + + +
4	Thin long rods; nonmotile; +ve	N + + + +; W + + + +; M.S. + + + +; V.D + + + + +	+
5	Short to medium rods motile; +ve	N + + +; W + + + −; M.S. + + +; V.D. + + +	+ + +
6	Thin long rods; nonmotile; −ve	N + + +; W + + +; M.S. + + +; V.D. + + +	+
7	Thick short rods; motile; +ve	N + + +; W + + +; M.S. + + +; V.D. + + +	+
8	Thick short rods almost of size of yeasts; nonmotile; −ve	N +; W +; M.S. + + +; V.D. + + +	+
9	Thin long rods with 3–4 granules in the cells; motile +ve	N + +; W + + +; M.S. + +; V.D. + + +	+
10	Thin long rods; nonmotile; −ve	N +; W + +; M.S. +; V.D + + +	+ +
11	Thin long rods; motile; +ve	N + +; W + + M.S. + +, V.D. + +	+
12	Thin rods motile (?) +ve	N + + +; W + + +; M.S. + + +; V.D. + + +	+ +
13	Short thin rod; motile; −ve	N + + +; W + + +; M.S. + + +; V.D. + + +	+
14	Medium thin rod; motile; −ve	N + + +; W + + +; M.S. + + +; V.D. + + +	+ + + +

* Short 0·5–2μ; Medium 2·0μ–4·0μ; Long 4μ upwards.

† N—Nutrient agar; W—Wort agar. M.S.—McGregor and Shene's agar; V.D.—Vandelden's sodium lactate-asperagin agar. + Slight growth; + + + + Profuse growth.

§ + Slight pink; + + + + Intense red.

(No H_2S produced by any culture; No thiosulphate utilised and H_2S not toterated.)
All cultures are aerophilic.

of H_2S by any one of the 14 cultures is probably due to the elimination of Saprophytes inherent to the methods of isolation described in the earlier portions of this paper. These Saprophytes are usually anærobes or microærophilis and perhaps therein lies the significance of the nitrate reducers which create a favourable environment for the saprophytes to thrive.

In the formation of elemental sulphur two well-defined stages appear to be involved. First, the production of H_2S by the deeper layers of the soil and second the oxidation of H_2S to yield sulphur. It is suggested that the reactive nitrite liberates sulphur from the H_2S thus formed—$KNO_2 + 3\ H_2S = KOH + 3\ S + NH_3 + H_2O$. The existence of powerful nitrite formers among the bacteria so far isolated lends significant support to the view that the above reaction may be operative in those strata where sulphur deposition occurs.

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1. Beijerinck, *Centralbl. f. Bakt., Abt. II*, Bd. 1. 1895, S.1, 49, 194 2. Van Delden, *Ibid.*, Bd. 11, 1904. S.81, 113. 3. Elion (Delft. Holland), *Ellis' Monograph on Sulphur Bacteria*, 1930.