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Production of Hydrogen Sulfide by Streptomycetes and Methods for its Detection

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ABSTRACT

KÜSTER, E. (University College, Dublin, Ireland), AND S. T. WILLIAMS. Production of hydrogen sulfide by streptomycetes and methods for its detection. Appl. Microbiol. **12**:46-52. 1964.—The ability of streptomycetes to produce hydrogen sulfide is generally used for taxonomic purposes. It was found that the previously used method, the blackening of Peptone Iron Agar, does not clearly indicate formation of hydrogen sulfide. It was shown that the blackening of a lead acetate strip is the most accurate indicator for H_2S-producing streptomycetes. A great variety of organic and inorganic sulfur compounds were examined and compared, and the choice of the most suitable sulfur source and method for the detection of hydrogen sulfide is discussed.

The capacity to produce hydrogen sulfide has been widely used as a diagnostic test for bacteria. However, this test was not generally applied to streptomycetes until the work of Tresner and Danga (1958), who studied a large number of strains with Peptone Iron Agar. Since this study, several workers have used hydrogen sulfide production as a test for streptomycetes (Turri and Silvestri, 1960; Prauser and Meyer, 1961; Pridham and Lyons, 1961; Sanchez-Marroquin, 1962). The results of Turri and Silvestri (1960) and Prauser and Meyer (1961) cast considerable doubt on the value of using Peptone Iron Agar as a means of detecting hydrogen sulfide production by streptomycetes. Evidence was obtained which indicated that this medium detected chromogenesis rather than hydrogen sulfide. The study reported here falls conveniently into two sections: first, the testing of various methods for detecting hydrogen sulfide; and second, an investigation of its production from various sulfur sources.

COMPARISON OF METHODS FOR DETECTING HYDROGEN SULFIDE PRODUCTION BY STREPTOMYCETES

The production of hydrogen sulfide by microorganisms is generally detected either by strips of lead acetate paper held at the mouth of culture tubes, or by incorporating into the culture medium salts of certain metals which form insoluble sulfides. The salts which have been most frequently used are those of lead, bismuth, and iron. In this study, media containing salts of these three metals were used in conjunction with lead acetate strips. Hydrogen sulfide production by 20 strains of streptomycetes (10 melanin-positive and 10 melanin-negative) from the collection of Kutzner (1956) was investigated.

Methods. Details of the media employed are as follows. Peptone Iron Agar was the dehydrated Difco product, containing: peptone, 15 g; proteose peptone, 5 g; ferric ammonium citrate, 0.5 g; dipotassium phosphate, 1 g; sodium thiosulfiate, 0.08 g; agar, 15 g; and distilled water, 1,000 ml. Lead Acetate Agar was prepared according to the formula of the Difco product of the same name: peptone, 15 g; proteose peptone, 5 g; dextrose, 1 g; lead acetate, 0.2 g; sodium thiosulfate, 0.08 g; agar, 15 g; and distilled water, 1,000 ml. Bismuth nitrate agar was prepared by the same formula as that given for lead acetate agar, the bismuth solution being substituted for lead acetate and added to give a concentration of bismuth nitrate of 0.4 g per liter. Most bismuth salts partially hydrolyze in water, giving precipitates of basic salts. However, if bismuth nitrate crystals are first triturated with mannitol, a clear solution is obtained when water is added. This solution becomes cloudy if it is autoclaved. Therefore, a mixture of bismuth nitrate (2 g) and mannitol (5 g) were dissolved in 100 ml of sterile water and portions of this solution were added to tubes of the basic medium which had been sterilized in the normal manner.

The pH of each medium was adjusted to 7.0 before sterilization.

The *Streptomyces* strains were inoculated onto slopes of these media, and a strip of lead acetate paper was inserted into each culture. The tubes were then incubated at 25 C for 7 days.

Positive control tubes for this experiment were prepared by passing chemically produced hydrogen sulfide into a tube of each medium until the metal sulfide was formed.

After the incubation period, formation of pigment in the medium and darkening of the lead acetate strips were noted. The culture and control tubes were then placed in a water bath, and the media were melted and boiled to drive off any excess hydrogen sulfide. While the media were still hot, a few drops of concentrated hydrochloric acid were added to each tube. The effect on the color of the media was noted, and any hydrogen sulfide evolved was detected by fresh strips of lead acetate paper, held at the mouth of the tubes. Thus, hot concentrated HCl dis-

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comes increasingly soluble at lower pH levels, while lead and bismuth sulfides remain insoluble at all values. The solubility of ferrous sulfide would be greater than that of lead or bismuth sulfide at the pH values around neutral which existed in these media. This was demonstrated by Hunter and Crecelius (1938), who found bismuth salts, incorporated into nutrient agar at pH 7.0, to be 20 times more sensitive than iron salts for detecting hydrogen sulfide.

In their investigations of Peptone Iron Agar, Turri and Silvestri (1960) used *Salmonella typhimurium* as a positive control. They found that this organism did not blacken the medium if it was inoculated onto the surface of a slope; if it was inoculated in stab culture, blackening was produced most strongly at the base of the stab, its intensity decreasing towards the surface. As previously stated, the positive controls used in our experiments were produced by passing H_2S into tubes of the three media. While the sulfides produced in the lead and bismuth media were stable, the black sulfide formed in sloped tubes of Peptone Iron Agar disappeared within 24 hr. The sulfide formed in unsloped tubes of this medium disappeared more slowly, the process commencing at the surface and gradually moving downwards until, after 1 week, the black color had completely gone. Thus, it would seem that the ferrous sulfide was oxidized by atmospheric oxygen, possibly to a ferric compound. This could explain the results obtained by Turri and Silvestri (1960) and provides another reason for the lack of sensitivity of Peptone Iron Agar.

The incorporation of lead and bismuth salts into media allowed detection of H_2S formation by streptomycetes, but each had certain disadvantages. (i) Both these metals are somewhat toxic, although no effects were observed with the strains used in these experiments. (ii) It is clear that on media containing potential melanin-forming substances the pigment produced by chromogenic strains masks the presence of the sulfides of these metals. Thus, H_2S production by chromogenic strains could only be verified by testing all pigments with concentrated HCl. (iii) Even on media lacking melanin-producing substances, the various nonmelanoid pigments produced by many *Streptomyces* strains would obscure, to a varying extent, the production of sulfides. (iv) While the lead sulfide produced by melanin-negative strains could be seen quite clearly, the bismuth sulfide did not diffuse very far into the medium, remaining very close to the regions of growth. Sometimes it was not until concentrated HCl was added to the tube that a positive strain was detected with certainty.

Thus, of the methods tested, only the lead acetate strips seemed to be satisfactory for use with streptomycetes. They are unaffected by pigmentation of the medium and can exert no toxic effects, and the positive reaction is clear and easily read. The use of lead acetate strips for bacterial cultures has been recommended by many workers. Zobell and Feltham (1934) found lead acetate papers to be ten times more sensitive than bismuth, lead, or iron salts incorporated into media; Fuchs and Bonde (1957), together with Mossel (1962), noted that lead acetate strips were much more sensitive than iron salts. Lead acetate on strips was also found to be more sensitive than lead acetate in medium (Clarke, 1953; Buck and Cleverdon, 1960). Therefore, this method was employed in the subsequent experiments of this study.

Production of Hydrogen Sulfide From Various Sulfur Sources by Streptomycetes

Methods. To facilitate an evaluation of the diagnostic significance of the test, strains were chosen to represent a wide range of types. This was achieved by selecting strains from each of the ten groups in the classification scheme of Kutzner (1956), the number of cultures chosen from each group being roughly proportional to the total number placed in that group by Kutzner (Table 3). The strains selected included several species which were described as being "hydrogen sulphide negative or positive" by Tresner and Danga (1958).

Before commencing the investigation of individual sulfur sources, the 45 strains were inoculated onto slopes of Peptone Iron Agar to ascertain whether H_2S production from this medium (as assessed by the lead acetate strip method) had any diagnostic value. It was found that on this medium, which contains three potential sulfur sources (peptone, proteose peptone, and sodium thiosulfate), all 45 strains blackened the strips after 4 days of incubation at 25 C. Thus, Peptone Iron Agar appeared to be of little use for the separation of *Streptomyces* strains on the basis of hydrogen sulfide production. Once more, it was noted that only chromogenic strains produced a blue-black pigmentation in this medium.

Sulfur-containing substances selected as sulfur sources represented a range of chemical structure. Three inorganic sulfur compounds, sodium sulfate, thiosulfate, and sulfite, were used. Organic compounds with a relatively simple structure were represented by the three amino acids, L-cysteine, L-cystine, and L-methionine, and by thiourea. More complex organic substances were represented by

TABLE 3. *Selection of strains from Kutzner's groups*

Group	No. of strains
I	13
II	4
III	1
IV	2
V	2
VI	9
VII	9
VIII	2
IX	2
X	1
Total	45

solved the sulfides of iron, lead, and bismuth, with evolution of hydrogen sulfide (Table 1). This was a convenient method by which to verify the formation of sulfides in the three media. In addition, the three media described above were prepared with the omission of the H_2S-detecting substances. The strains were inoculated onto these media, lead acetate strips were inserted, and, after the standard incubation period, the formation of pigments and darkening of the strips were noted. The color and occurrence of these pigments and the reactions of the strips were then compared with those obtained when H_2S detectors were included (Table 2).

Results. From Tables 1 and 2, the following points are evident.

(i) Hydrogen sulfide was liberated from Peptone Iron Agar by all strains, as shown by the lead acetate strips. However, no pigment was produced in this medium by melanin-negative strains and the blue-black pigment in the chromogenic cultures did not contain sulfide. Thus, the ferric salt in this medium did not detect the hydrogen sulfide produced by these strains. The blue-black pigment produced by some strains in this medium is clearly an indication of chromogenesis rather than hydrogen sulfide. It is clear (Table 2) that the presence of the iron salt did influence the color of the pigment formed by melanin-positive strains; in the presence of iron it was a bluish-black color, whereas without iron a brownish pigment was formed. A similar effect was noted by Turri and Silvestri (1960) and Prauser and Meyer (1961); the former investigators concluded that the blue-black pigment was formed by the reaction of ferric ions and aromatic compounds containing hydroxyl groups.

(ii) All strains liberated hydrogen sulfide from the lead acetate agar, as indicated by the lead acetate strips. The pigment produced in this medium by melanin-negative strains gave the same reaction with concentrated HCl as did the positive control, thus indicating that it was pure lead sulfide. The brown pigment produced by melanin-positive strains was apparently a mixture of lead sulfide and melanin, as it was only partially dissolved by concentrated HCl. As can be seen from Table 2, the pure melanin pigment, produced by chromogenic strains in the absence of lead acetate, was indistinguishable in color from that formed in the presence of the lead salt.

(iii) No darkening of the lead acetate strips was produced by cultures grown on bismuth nitrate agar. It was clear, from the testing of pigments formed in this medium, that bismuth sulfide was formed. Thus, it seemed that all the hydrogen sulfide produced was incorporated into bismuth sulfide, none being released from the medium. Again, the pigment produced by melanin-positive strains was a mixture of the sulfide and melanin, this being identical in color with the pure melanin pigment (Table 2).

Discussion. The lack of sensitivity of the ferric salt in Peptone Iron Agar can be partially explained by the chemical properties of the sulfides. Ferrous sulfide be-

TABLE 1. *Methods for detecting hydrogen sulfide production by streptomycetes*

Culture	Determination	Peptone Iron Agar	Lead Acetate Agar	Bismuth nitrate agar
Melanin-negative strains	Darkening of lead acetate strips during growth of cultures	+	+	−
	Pigmentation produced in medium	None	Brown	Brown
	Solubility of pigment in concentrated HCl, with evolution of H_2S	−	+	+
Melanin-positive strains	Darkening of lead acetate strips during growth of cultures	+	+	−
	Pigmentation produced in medium	Blue-black	Brown to orange-brown	Brown to orange-brown
	Solubility of pigment in concentrated HCl, with evolution of H_2S	−	±	±
Control tubes	Pigmentation produced in medium	Black	Brown	Brown
	Solubility of pigment in concentrated HCl, with evolution of H_2S	+	+	+

TABLE 2. *Results obtained by use of media with and without H_2S-detecting substances*

Medium	Melanin-negative strains		Melanin-positive strains	
	Pigments in medium	Reaction of lead acetate strips	Pigments in medium	Reaction of lead acetate strips
Peptone Iron Agar	—	+	Brown to orange-brown	+
Peptone Iron Agar plus Fe-ammonium citrate	—	+	Blue-black	+
Lead Acetate Agar	—	+	Brown to orange-brown	+
Lead Acetate Agar plus lead acetate	Brown	+	Brown to orange-brown	+
Bismuth nitrate agar	—	+	Brown to orange-brown	+
Bismuth nitrate agar plus bismuth nitrate	Brown	−	Brown to orange-brown	−

thiamine, with sulfur incorporated into a ring structure, and by egg albumen, a protein rich in sulfur. In addition, three complex protein hydrolysates produced by Difco (peptone, proteose peptone, and Tryptone) were used.

The sulfur-containing substances were incorporated into a basal medium of the following composition: glycerol, 10 g; L-asparagine, 1 g; dipotassium phosphate, 1 g; sodium chloride, 2 g; magnesium chloride, 0.5 g; calcium carbonate, 0.2 g; and distilled water, 1,000 ml.

In the cases of peptone, proteose peptone and Tryptone, only dipotassium phosphate and sodium chloride were added. The pH of each medium was adjusted to 7.0, by use of sodium hydroxide, before sterilization.

Before studying the effect of the various sulfur sources, all strains were inoculated onto the basal medium containing no known source of sulfur. It was found that, although no hydrogen sulfide was evolved by strains growing on the liquid basal medium, several were positive when growing on the basal medium to which agar had been added. When Difco agar was used, 21 of the 45 strains gave a positive reaction; when Oxoid agar No. 3 was substituted, 7 strains produced hydrogen sulfide. Even Ionagar No. 2 (Oxoid), although highly purified, showed a positive H_2S reaction by six *Streptomyces* strains. One of the strains, *S. coelicolor*, gave such a strong reaction that all the results on solid media had to be disregarded. Oxoid agar No. 3 was selected for use in the subsequent experiments. None of the positive strains appeared to liquefy agar, so it would seem that the hydrogen sulfide was produced from traces of contaminating substances rather than from the sulfur present in the polysaccharide chains of agar. The different results obtained with the three types of agar also supported this view.

Both solid and liquid media were prepared with each sulfur source, with the exception of egg albumen. Each substance was added to the basal medium to give a final concentration of approximately 0.1 g of sulfur per liter of of medium. The substances were added to the medium before sterilization, with the exception of the amino acids, thiamine, and thiourea. These three substances were dissolved in 100 ml of sterile water (10% HCl in the case of cystine) and 1-ml portions of the solutions were added to tubes of the sterilized basal medium. This procedure, which was basically the same as that use by Pridham (*unpublished data*) in dealing with thermolabile sugars and amino acids, caused no contamination.

To detect H_2S production, the 45 streptomycetes were inoculated onto the solid and liquid media prepared for each sulfur source. Strips of lead acetate paper were inserted, and the cultures were incubated for 14 days at 25 C. Blackening of the strips was noted at intervals throughout this period. As hydrogen sulfide production from some sources was relatively slow and from others nonexistent after 14 days, strains on such sources were incubated for an additional 14 days before the final reading was made. In all cases, uninoculated control tubes were also incubated to facilitate detection of any hydrogen sulfide produced nonbiologically.

Results. In Table 4, details of the final figures for hydrogen sulfide production from the various sulfur sources are given. In the last column, the overall percentage of positive strains is shown; this was obtained by combining the results from the solid and liquid media. Thus, for example, one strain was negative on solid methionine medium (giving 98% positive) but this strain was positive on liquid methionine medium, so the combined figure for this source was 100%. An indication of the time needed for hydrogen sulfide production from the various sources is given in Table 5. From these results, the following points are evident.

(i) With exception of thiourea, thiamine, and egg albumen, the organic sulfur compounds yielded a very high percentage of positive strains. As the main sources of sulfur in the protein hydrolysates are normally assumed to be L-cysteine and L-cystine, it was not surprising that the results for these sources were similar to those for the amino acids. The percentage of positive strains of liquid media containing peptone, proteose peptone, and Tryptone were somewhat lower than those for the corresponding solid media. This was probably due to the slower growth of many strains in liquid media containing these substances. The sulfur in thiourea and thiamine was apparently unavailable for reduction by any of the strains used, no hydrogen sulfide being detected even after 28 days of incubation. Some strains gave a positive reaction on egg albumen, but production of hydrogen sulfide was very slow, and generally only a faint darkening of the strips was noted.

(ii) The percentage of positive strains on inorganic sulfur sources was somewhat lower, ranging from 65% on sulfate to 91% on thiosulfate.

TABLE 4. *Production of hydrogen sulfide from various sulfur sources by streptomycetes*

Time of incubation*	Sulfur source	Per cent positive strains on		Combined per cent positive strains
		Solid medium	Liquid medium	
days				
14	L-Cysteine	100	100	100
	L-Cystine	100	84	100
	L-Methionine	98	100	100
	Peptone	100	82	100
	Proteose peptone	98	73	98
	Tryptone	100	64	100
28	Thiourea	0	0	0
	Thiamine	0	0	0
	Egg albumen	41	—	41
	Sodium sulfite	75	79	84
	Sodium thiosulfate	80	84	91
	Sodium sulfate	45	49	65

* Incubation was at 25 C. Results were recorded after the indicated number of days.

(iii) The time elapsing before the production of hydrogen sulfide varied with the type of sulfur source and the physical state of the medium. Generally, production occurred more quickly from organic than from inorganic sulfur compounds, the production from L-cysteine being particularly rapid. Production from liquid media generally lagged behind that from the corresponding solid medium, reflecting the slower growth rate of most strains in the former. On solid media containing organic sulfur compounds, a large percentage of strains were positive after 2 to 3 days; on liquid media, most were positive after 6 to 10 days. On solid media containing inorganic sulfur sources, few new positive strains were detected after 10 to 14 days of incubation; on the liquid media, there was a steady increase up to 28 days.

(iv) The degree of blackening of the strips, produced by strains growing on the organic compounds with available sulfur, was generally very marked; with inorganic sources, the strength of the reaction was more variable but still clear and easily read. It was noticed, when cultures were kept for 28 days, that the blackening of the strips gradually faded until, in some cases, it disappeared almost completely.

(v) The replication between the results from solid and liquid media containing inorganic sulfur compounds is indicated in Table 6. Results from the solid and liquid media were in best agreement when sodium sulfite was used; only 14% of the strains gave a differing reaction. When sodium sulfate was used, 36% of the strains gave different reactions on the solid and liquid media.

Discussion. A high percentage of strains produced hydrogen sulfide from the three amino acids. It is well known that many bacteria evolve hydrogen sulfide when grown on media containing L-cysteine. Vaughn and Levine (1936) concluded that a positive reaction with this amino acid had no value for differentiating bacteria of the colon group, and nearly all of the strains studied by Clarke (1953) gave a positive reaction with this source. Mossel (1962) found that all Enterobacteriaceae were positive on this substrate. Prauser and Meyer (1961) recommended a cysteine-bismuth medium for studying hydrogen sulfide production by streptomycetes. The results obtained in our work indicated that, when a sensitive detection method is used, the formation of hydrogen sulfide from cysteine by streptomycetes is of little or no diagnostic significance. Several pathways, by which microorganisms may form sulfide from cysteine, have been postulated. The most clearly defined path is catalyzed by a pyridoxal phosphate-dependent desulfhydrase which was isolated from *Escherichia coli* by Metaxas and Delwiche (1955).

Results obtained with L-cystine and L-methionine were similar to those for L-cysteine. Fromageot (1951) suggested that cystine was first reduced to cysteine before hydrogen sulfide was produced. This offers a possible explanation for the slight lag (2 to 3 days) in the formation of sulfide from cystine as compared with cysteine, which was apparent in our results (Table 5). Formation of hydrogen sulfide from L-methionine by streptomycetes was also observed by Turri and Silvestri (1960).

The various preparations of protein hydrolysates have often been used in media designed to assess hydrogen sulfide formation by microorganisms. The three used in these tests, peptone, proteose peptone, and Tryptone, all yielded a high percentage of positive strains, particularly in solid media.

Thus, none of the organic sulfur compounds tested seemed to be of much value for the diagnosis of streptomycetes; either no hydrogen sulfide was produced (traces in the case of egg albumen) or nearly all strains gave a positive reaction. The figures obtained with liquid media containing peptone, proteose peptone, and Tryptone were more intermediate. However, a high percentage of strains were able to produce hydrogen sulfide from these sources in solid media, and growth of many strains was very poor in the liquid cultures. Thus, the figures were more a reflec-

TABLE 5. *Time needed for production of hydrogen sulfide from various sulfur sources*

Sulfur sources	Medium*	Time (days)								
		1	2	3	4	6	10	14	21	28
L-Cysteine	S	89	96	100						
	L	53	58	60	80	94	100			
L-Cystine	S	9	57	85	96	100				
	L	0	4	20	53	73	82	84		
L-Methionine	S	45	84	91	96	98	98	98		
	L	0	22	53	80	93	98	100		
Tryptone	S	20	91	96	100					
	L	0	20	31	31	44	60	64		
Peptone	S	41	91	91	96	98	100			
	L	0	0	18	47	62	67	82		
Proteose peptone	S	20	86	91	98	98	98	98		
	L	0	4	18	22	42	56	73		
Egg albumen	S	0	0	0	0	0	2	25	39	41
	L									
Sodium thiosulfate	S	0	7	20	43	57	66	75	77	80
	L	0	2	13	18	24	36	58	69	84
Sodium sulfate	S	0	0	0	0	4	16	36	43	45
	L	0	0	0	0	18	22	33	38	49
Sodium sulfite	S	0	30	57	47	66	73	73	75	75
	L	0	0	4	13	44	51	62	71	79

* L = liquid medium; S = solid medium.

TABLE 6. *Production of hydrogen sulfide from solid and liquid media containing inorganic sulfur compounds**

H_2S production from		Percentage of strains on		
Solid medium	Liquid medium	Sodium sulfite	Sodium thiosulfate	Sodium sulfate
+	−	5	7	16
−	+	9	11	20
+	+	70	73	29
−	+	16	9	35

* Results after 28 days at 25 C.

tion of difference of growth rate than of the capacity to produce hydrogen sulfide.

Many of the strains tested were able to form hydrogen sulfide from inorganic sulfur compounds. Formation of sulfide from sulfite and thiosulfate by bacteria was observed by several workers. Clarke (1953) noted that production from sodium thiosulfate was slower and weaker than from L-cysteine or L-cystine. Formation of sulfide by *Clostridium perfringens* from sulfite, thiosulfate, cysteine, and cystine was observed by Fuchs and Bonde (1957). Artman (1956) noted that *E. coli* formed sulfide from thiosulfate, but not from sulfate or sulfite. All the strains of Enterobacteriaceae tested by Mossel (1962) produced sulfide from sulfite and thiosulfate. The enzymes concerned in these reactions were elucidated in certain cases. A reduced nicotinamide adenine dinucleotide phosphate-specific sulfite reductase, reducing sulfite to sulfide, was found in extracts of *E. coli* by Mager (1960), and Kaji and McElroy (1959) isolated an enzyme from yeast which catalyzed the reductive cleavage of thiosulfate to sulfite and hydrogen sulfide.

Production of hydrogen sulfide from sulfate is not normally associated with aerobic heterotrophic microorganisms, which are thought to carry out assimilatory sulfate reduction with sulfate as a sole sulfur source but not forming detectable amounts of the gas. Large-scale reduction of sulfate to sulfide is carried out by the sulfate-reducing bacteria, which use sulfate as the terminal electron acceptor in anaerobic respiration. However, it is clear that certain streptomycetes could produce appreciable amounts of hydrogen sulfide from sulfate under aerobic conditions. A similar result was obtained by Bromfield (1953), who noted that *Bacillus megaterium* reduced sulfate to sulfide in soil under aerobic conditions.

The percentages of strains capable of producing hydrogen sulfide from inorganic sulfur sources were somewhat lower than those obtained with most organic compounds. The figures obtained with sodium sulfite and thiosulfate were still rather too high to be of diagnostic value. The results with sodium sulfate came nearest to the desirable ratio of 1:1 for positive and negative strains. However, production of hydrogen sulfide from sulfate was much slower than from sulfite and thiosulfate, and the reaction was generally much weaker. Also, the replication between solid and liquid media containing sulfate was poor. Further studies with sodium sulfate revealed that many strains did not give the same reaction in replicate experiments. It is possible that, during the long incubation period needed, autolytic processes may have resulted in some hydrogen sulfide production.

It was hoped that the data obtained from these experiments would facilitate the selection of a set of conditions under which this test could be applied to streptomycetes. This, however, proved to be a very difficult task. Many of the sulfur sources tested gave a very high percentage of positive strains if cultures were incubated until no further positive reactions were detected. Other sources either gave very slow, weak production of hydrogen sulfide, or none was detected. A lower percentage of positive strains could be obtained by curtailing the incubation period, but the results would be of doubtful taxonomic value, and slight changes of the inoculum size and other experimental conditions would tend to produce nonreplicate results.

Similarly, the choice between liquid and solid media is difficult. When liquid media were used, there was great variety in the extent and pattern of growth by different strains; some produced very little growth even after 28 days and, hence, were usually negative for hydrogen sulfide production. On solid media, good growth was made by all strains after 1 week, and the amount of growth was similar. However, the ability of several strains to produce hydrogen sulfide from agar was a serious disadvantage encountered when using solid media.

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LITERATURE CITED

ARTMAN, M. 1956. The production of hydrogen sulphide from thiosulphate by *E. coli*. J. Gen. Microbiol. **14**:315–322.

BROMFIELD, J. M. 1953. Sulphate reduction in partially sterilized soil exposed to air. J. Gen. Microbiol. **8**:378–390.

BUCK, J. D., AND R. C. CLEVERDON. 1960. Hydrogen sulphide production by some agarolytic marine bacteria. Can. J. Microbiol. **6**:594–595.

CLARKE, P. H. 1953. Hydrogen sulphide production by bacteria. J. Gen. Microbiol. **8**:397–407.

FROMAGEOT, C. 1951. Desulfhydrases, p. 1237–1243. *In* J. B. Sumner and K. Myrbäck [ed.], The enzymes vol. 1, part 2. Academic Press, Inc., New York.

FUCHS, A. R., AND G. J. BONDE. 1957. The availability of sulphur for *Clostridium perfringens* and an examination of hydrogen sulphide production. J. Gen. Microbiol. **16**:330–340.

HUNTER, C. A., AND H. G. CRECELIUS. 1938. Hydrogen sulphide studies. I. Detection of hydrogen sulphide in cultures. J. Bacteriol. **35**:185–196.

KAJI, A., AND W. D. MCELROY. 1959. Mechanism of hydrogen sulfide formation from thiosulfate. J. Bacteriol. **77**:630–637.

KUTZNER, H. J. 1956. Beitrag zur Systematik und Ökologie der Gattung Streptomyces Waksm. et Henr. Dissertation, Landwirtschaften Hochschule, Hohenheim, Germany.

MAGER, J. 1960. A TPNH-linked sulfite reductase and its relation to hydroxylamine reductase in Enterobacteriaceae. Biochim. Biophys. Acta **37**:314.

METAXAS, M. A., AND E. A. DELWICHE. 1955. The L-cysteine desulfhydrase of *Escherichia coli*. J. Bacteriol. **70**:735–737.

MOSSEL, D. A. A. 1962. Formation of hydrogen sulphide from substrates by groups of bacteria frequently encountered in perishable food materials. Ann. Inst. Pasteur **103**:108–111.

PRAUSER, H., AND J. MEYER. 1961. Zum Nachweis der H_2S-Bildung bei Streptomyceten. Naturwissenschaften **48**:463.

PRIDHAM, T. G., AND A. J. LYONS. 1961. *Streptomyces albus* (Rossi-Doria) Waksman et Henrici: taxonomic study of strains labeled *Streptomyces albus*. J. Bacteriol. **81**:431–441.

SANCHEZ-MARROQUIN, A. 1962. Constancy of characteristics in the streptomycetes. J. Bacteriol. **83**:1183–1192.

TRESNER, H. D., AND F. DANGA. 1958. Hydrogen sulfide production by Streptomyces as a criterion for species differentiation. J. Bacteriol. **76**:239–244.

TURRI, M., AND L. SILVESTRI. 1960. Osservaziono su un saggio biochimico proposto per la classificazione degli Actinomiceti. Ann. Microbiol. Enzimol. **10**:71–76.

VAUGHN, R., AND M. LEVINE. 1936. Hydrogen sulfide production as a differential test in the colon group. J. Bacteriol. **32**:65–73.

ZOBELL, C. E., AND C. B. FELTHAM. 1934. A comparison of lead, bismuth, and iron as detectors of hydrogen sulphide produced by bacteria. J. Bacteriol. **28**:169–176.