VARIATIONS IN HYDROGEN SULPHIDE PRODUCTION BY BACTERIA

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In a paper already published (1921) the writer has called attention to the effect of peptone on indol production. In the present paper there are reported experiments dealing with the effect of peptone on the production of hydrogen sulphide by bacteria.

EXPERIMENTAL WORK

The culture medium employed in nearly all of the experiments was lead acetate agar prepared according to the directions given by Jordan and Victorson (1917), with some modifications, as follows: 3 per cent of peptone was dissolved by boiling in fresh meat infusion; after filtration 1.5 per cent agar was added and dissolved by heating in the autoclave, the reaction was adjusted to pH 7.2 and the medium tubed and sterilized in the autoclave. After sterilization the tubes were cooled to 43°C. and 3 drops of a freshly made 10 per cent solution of lead acetate added to each tube. The tubes were then shaken vigorously and rapidly cooled. The cultures used were grown in peptonized beef broth and the lead acetate tubes were each inoculated with one loopful of broth culture, the loop being introduced between the agar and the wall of the tube and passed down to the bottom. It was found that the best results were obtained when young broth cultures were used for inoculating the agar; twenty-four hour cultures were usually employed, but forty-eight-hour cultures gave results just about as good and were used when more convenient.

The results of a number of such experiments are given below in table 1. Numbers are used to indicate the different varieties of peptone while letters with the numbers indicate different samples of the same varieties of peptone.

Table 1 shows that there are decided differences between the different peptones and between different samples of the same

TABLE 1

Comparative hydrogen sulphide formation with different peptones

Experiment 1

CULTURES	PEPTONES				
	1A	2	3	4A	5
Proteus 4	−	+++	−	−	++
Proteus 19	−	+++	−	−	++
Bact. suipestifer 360	−	−	−	−−	−
Bact. suipestifer 416	−	+++	−	+++	++
Bact. typhosum A	−	+++	−	+++	+++
Bact. typhosum D	−	−	−	−	−

Experiment 2

CULTURES	PEPTONES				
	1B	1C	1D	4B	6
Proteus 4	+	+	++	+++	+++
Proteus 19	+	+	++	+++	+++
Bact. suipestifer 360	−	−	−	−	−
Bact. suipestifer 416	+	−	++	+++	+++
Bact. typhosum A	+	+	++	+++	+++
Bact. typhosum D	−	−	−	−	−

− = Negative reaction. + = Weak reaction. ++ = Good reaction. +++ = Strong reaction.

peptone. It also shows that different strains of the same organism may produce different results.

The differing results with different strains of the same organism seemed to warrant more study and as a result there was discovered a curious correlation between hydrogen sulphide production in lead acetate agar and growth in a synthetic medium having the following composition:

Sodium ammonium hydrogen phosphate.....0.2 per cent
Glucose.....1 per cent
Brom cresol purple.....5 drops of 0.04 per cent solution per tube

The reaction was not adjusted. The medium was sterilized in ordinary bacteriological tubes at 15 pounds pressure for fifteen to twenty minutes. Growth caused acid production and a change in color from purple to yellow.

TABLE 2

Hydrogen sulphide production by different strains of Bact. typhosum and Bact. suipestifer

ORGANISM	H_2S REACTION	SYNTHETIC MEDIUM
Bact. suipestifer		
Number 320	Positive	Growth
Number 334	Negative	No growth
Number 346	Negative	No growth
Number 360	Negative	No growth
Number 407	Positive	Growth
Number 416	Positive	Growth
Number 420	Positive	Growth
Number 486	Negative	No growth
Bact. typhosum		
A	Positive	No growth
HL	Positive	No growth
C	Positive	No growth
D	Negative	Growth
K 110	Negative	Growth
K 111	Positive	No growth
K 114	Positive	No growth

The correlation above mentioned is shown in table 2, which gives the consolidated results of a number of experiments. The peptone used in the lead acetate agar was peptone 2; the results under synthetic medium are those obtained after twenty-four hours' incubation; the results under H_2S reaction represent observations made after one to three days' incubation.

The correlation shown above between the ability of bacteria to produce H_2S and their ability to grow in the synthetic medium appears to be an interesting coincidence rather than evidence of any fundamental connection between the two phases of bacterial

metabolism concerned. As the table shows, the relation between H_2S formation in lead acetate agar and ability to grow in the synthetic medium is exactly opposite in the case of *Bact. typhosum* to what it is in the case of *Bact. suipestifer*. In addition it may be stated that three strains of *Bact. coli* all failed to produce H_2S and all grew in the synthetic medium three strains of *Bact. dysenteriae* all failed to produce H_2S and two failed to grow in the medium while the third grew very slowly, four strains of *Bact. enteritidis* all produced H_2S and all grew in the synthetic medium, eight strains of Proteus all produced H_2S and all grew in the synthetic medium, although three were slow in developing, and 15 strains of *Bact. paratyphosum* B all produced H_2S and all grew promptly in the synthetic medium.

It will thus be seen that although ability to produce H_2S and ability to grow in the synthetic medium are not related in a uniform way in the different species of organisms mentioned above, the relation between them is quite constant so far as any one species is concerned.

This is by no means an invariable rule, since exceptions to it are frequently observed. But as far as *Bact. suipestifer* is concerned, out of more than 150 strains recently examined in this division, only 6 failed to conform to the rule that strains which produce H_2S also grow in the synthetic medium while those which do not produce H_2S do not grow in the synthetic medium.

In regard to the relation between hydrogen sulphide production by bacteria and the chemical composition of the various peptones it may be stated that the writer has attempted in connection with the work herein recorded to follow the example of his previous work upon indol production, where everything depended upon the presence or absence of tryptophan, and show that the relative value of peptones for hydrogen sulphide production depended upon the amount of cystin present. It was soon found, however, that the problem was not as simple as it seemed and it was therefore made the subject of a new line of inquiry, the results of which the writer hopes to present before long in another paper.

It can be stated at the present time, however, that experiment has shown that the addition of cystin to lead acetate agar made

with peptone 1 causes the production of H_2S by *Bact. coli* and other organisms which are usually lead negative; while on the other hand the addition of a small amount of sodium thiosulphate makes it possible to obtain results similar in every way to those obtained with peptone 2.

DISCUSSION

The fact that different peptones will yield different amounts of hydrogen sulphide has previously been mentioned by Myers (1920) and by Thompson (1920–21). Each of them used Difco, Fairchild and Witte peptones, which are also included among the 6 used by the present writer. Myers used fluid media while Thompson used a lead acetate agar prepared by a method differing somewhat from that used by the writer. Neither of them has reported any difference between different strains of the same organism so far as H_2S production is concerned. In the case of *Bact. suipestifer* it seems likely that these differences between strains are the cause of conflicting statements in the literature. For example, according to Jordan and Victorson (1917) *Bact. suipestifer* does not produce hydrogen sulphide, while according to Buchanan (1916) and McFarland (1919) it does produce hydrogen sulphide.

The writer has designated the various peptones by numbers and letters instead of giving names, because his object was not to show the superiority or inferiority of any particular varieties of peptones but rather to show what a variable quantity "peptone" really is,—so variable, indeed, that if the names of the peptones were given the information would be more likely to mislead the reader than to help him.

As evidence of this it may be noted that Myers and Thompson ascribe different relative merits to the 3 peptones used by them, while the present writer's work would indicate still different relative values. What else could be expected if we stop to think that, after all, "peptone" in the bacteriological sense of the word is only a name for an indefinite mixture of proteoses, peptones, polypeptides and various other compounds varying enormously

in composition, depending on the materials used and the method of manufacture?

It would seem that until such time as we possess more exact knowledge of the composition of our culture materials, correct results will be possible only if all new culture materials are tested before use and found to be suitable for the particular work to be done.

CONCLUSIONS

1. When used in lead acetate agar different peptones now available in this country yielded extremely variable results so far as hydrogen sulphide production is concerned. In some instances different samples of the same peptone differed from each other.

2. The different strains of *Bact. suipestifer* and *Bact. typhosum* were found to be divided into two groups, those in one group producing hydrogen sulphide while those in the other group failed to produce hydrogen sulphide.

3. On account of variation in the composition of commercial peptones and variation in the hydrogen sulphide producing power of different strains of the same organism, it is advisable before using new culture materials in work with unknown organisms to test these culture materials with known strains of known organisms.

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