FERMENTATION OF SUGARS BY BACTERIUM TULARENSE

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The writer has reported fermentation of glucose, levulose, maltose, mannose and glycerol by *Bacterium tularense* with production of acid but no gas, in several publications 1926, 1927, 1928 and 1939. My first positive results were obtained with four strains in 1923 and have now been extended to 60 strains. The above results were confirmed in regard to 21 strains by Downs and Bond (1935) who also added maltose which I had not previously reported as a fermentable substance. Failure to obtain fermentation of glucose was reported by Shaw (1930) who tested only two strains. Failure to obtain fermentation of any sugars including glucose, levulose, maltose, mannitol, and also glycerol, was reported by Vereninova (1938) using 20 strains of *B. tularense.*

Bacterium tularense is a difficultly cultivatable microorganism. It fails to grow on ordinary laboratory culture mediums. A dry, solid, slanted surface is one of the necessary conditions for obtaining abundant growth. No liquid medium is suited to its growth, and for this reason the usual method of testing bacteria for fermentation by inoculating a liquid or semi-solid medium containing the fermentable substance is an impracticable procedure.

My work and that of Downs and Bond employed the solid, slanted surface in test tubes, as of my blood-glucose-cystine agar medium, but modified by substituting horse serum for rabbit blood, by substituting one of the fermentable substances for glucose, and by adding brom-thymol-blue as an indicator. Fermentation with acid production is indicated by a change of color of the medium from green to yellow. The non-fermentable sub-

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stances do not effect a color change but the color remains green as in the control culture tubes to which no sugar has been added. Final results were recorded after 7 days' incubation at 37°C. A completely yellow color of the growth and of the medium was recorded as ++++. If only a trace of green remained, the reaction was recorded as +++. Absence of any yellow color was recorded as 0.

Detailed instructions for making the culture medium are as follows: Fresh beef infusion agar, containing 1.0 per cent peptone, 1.5 per cent agar, and 0.5 per cent sodium chloride, adjusted to a pH of 7.3, is kept on hand in stock. Agar medium containing a large amount of sediment is preferable to the usual very clear agar. When needed, there is added to the stock agar 0.1 per cent of cystine, and this is heated in an Arnold steam sterilizer sufficiently long to melt the agar and to sterilize the cystine.

The addition of cystine does not change the pH of the medium, but if cysteine hydrochloride is substituted for cystine a correction may be necessary on account of acidity. Cystine is not very soluble in the beef infusion peptone agar and for that reason it should be pulverized before being added; even then, visible particles settle in the medium. Complete solution of the cystine may be obtained by frequent shaking of the flask while in the Arnold sterilizer, followed, if necessary, by allowing the melted cystine agar to remain over night in a 56°C. water bath.

To the flask (usually containing a liter of melted medium) is added the sterile indicator, brom-thymol-blue, sufficient to give a marked green color. The contents of the flask are divided into 10 small flasks, each containing 100 ml. of medium, and to each is added 1.0 per cent of the fermentable substance in solution which has been previously sterilized by Berkefeld filtration. The ten flasks (kept at 56°C.) are ready for tubing and slanting, but at the moment before tubing, 5 ml. of sterile horse serum are added to each 100 ml. flask of medium. The slanted tubes are incubated three days for sterility and are then ready for inoculation with a large loopful of growth scraped from the slanted surface of a stock tube of blood-glucose-cystine agar.

Table 1 shows the fermentation reactions of 24 of 60 strains of

Bacterium tularense which I studied. Acid was produced, but no gas. In isolated instances the reaction swung to the alkaline side. Of 60 cultures studied, all fermented glucose, maltose and mannose; 53 fermented glycerol, but 7 did not; 17 fermented levulose but 43 did not; dextrine was fermented more or less by about half of the cultures. None fermented mannitol, galactose, xylose,

TABLE 1

Fermentation Reactions of 60 Strains of Bacterium tularense

STRAINS	PLACE OF ISOLATION	YEAR OF ISOLATION	GLUCOSE, 60 POS.	LEVULOSE, 17 POS., 43 NEG.	GLYCEROL, 53 POS., 7 NEG.	MALTOSE, 60 POS.	MANNOSE, 60 POS.
Russ	Russia	1928	++++	++++	0	++++	++++
Max	Russia	1928	++++	++++	0	++++	++++
H.D.	Austria	1935	++++	+++	0	++++	++++
Jap	Japan	1926	++++	++++	0	++++	++++
Ohara	Japan	1931	++++	++++	0	++++	++++
Perry	California	1927	++++	++++	0	++++	++++
Sn	Montana	1925	++++	+++	0	++++	++++
38	Utah	1921	++++	0	++++	++++	++++
Mar	Washington, D. C.	1923	++++	0	++++	++++	++++
D.	Minnesota	1926	+++	0	++++	++++	++++
L R	Arkansas	1927	++++	0	++++	++++	++++
Va	Virginia	1931	++++	0	++++	++++	++++
Omo	Virginia	1931	++++	0	++++	++++	++++
Broo	New York	1941	++++	0	++++	++++	++++
R R P	Montana	1929	++++	++++	++++	++++	++++
Col	South Carolina	1932	++++	++	++++	++++	++++
Sea	Washington	1935	++++	+++	++++	++++	++++
Die	California	1938	++++	+++	++++	++++	++++
A 1	Arizona	1925	++++	+++	++++	++++	++++
V R 1	Washington, D. C.	1924	++++	++	++++	++++	++++
26	Utah	1921	++++	++	++++	++++	++++
12	Utah	1921	++++	++	++++	++++	++++
S.F.	California	1922	++++	++	++++	++++	++++
T	Montana	1923	++++	++	++++	++++	++++

trehalose, salicin, arabinose, adonitol, sucrose, lactose, amygdalin, dulcitol, erythritol, inositol, inulin, raffinose, sorbitol, or rhamnose.

DISCUSSION

Fermentation reactions are reported on 60 strains of *Bacterium tularense*, 55 of which were isolated in the United States, 2 in

Japan, 2 in Russia, and 1 in Austria. The reason for including in the Table the detailed results of only 24 of the 60 strains which I tested is that the 24 selected strains amply demonstrate the differences of fermentation reactions found in the 60 strains. Those differences relate to the action on levulose and glycerol.

In regard to glycerol, special interest attaches to the small number of strains which failed to ferment glycerol—only seven—and of these five were of foreign origin. In regard to levulose, only 17 of 60 strains fermented this sugar and these include the seven strains which did not ferment glycerol.

Because of the ready fermentation of glucose by *Bacterium tularense* this sugar was incorporated into my stock culture medium. Fermentation means utilization by the organism and consequent increased volume of growth. The fermentation of glycerol, mannose, and maltose, bear the same suggestion of growth promotion and these might be important constituents of a stock culture medium.

Classification of *Bacterium tularense* cultures on a basis of sugar fermentations is impracticable.

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