XCIII. THE SYNTHESIS OF STARCH FROM SUGAR BY BACTERIA.

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GRANULES staining blue with iodine have been noticed in higher fungi, bacteria and in protozoa, but the nature of the substance and its significance is not definitely known. The substance formed by *B. coli* is shown in the present communication to be no other than starch, and the writer is of the opinion that the proof of the existence of starch in bacteria is of the highest significance.

Starch is readily detected in the early stages of the fermentation of glucose in presence of chalk by bacteria. It is present in the solid bacterial debris, which settles together with the calcium oxalate precipitate when oxalic acid is added in the process of removing calcium after the fermentation, and it is present throughout the medium in the bacterial particles. After prolonged boiling with oxalic acid, in the process of distilling the volatile acids, it appears abundantly in the solution as soluble starch. It has been found present most abundantly from about the sixteenth to the fortieth hour of the fermentation. At some periods there appears to be an almost complete transformation of glucose into non-reducing carbohydrate, a great proportion of which has been shown to be starch. The solutions after removal of the various products of decomposition yield precipitates upon the addition of alcohol. The soluble starch gives the characteristic blue coloration with iodine, and shows a high dextrorotation, the extent of which depends upon the treatment by which the soluble starch has been formed from the original starch of the bacteria. Gentle treatment in the cold yields a soluble starch of very high rotation. Prolonged boiling with oxalic acid yields a soluble starch or amyloextrin showing a dextrorotation, $[\alpha]_D = 200^\circ$. The soluble starch of highest rotation showed no reduction with copper solutions. Samples of lower rotation showed in some cases reduction. It yields glucose on hydrolysis. I have not so far been able to find any glycogen.

The discovery of the production of vegetable starch by bacteria establishes still further my thesis that the fermentation processes of many different organisms are *au fond* very similar, a thesis which I desire to maintain because I believe that many physiological problems will be more readily solved by the study of the so-called lower organisms than the higher. I have for some time tried to emphasise the importance of the synthetic side of bacterial fermentation, and I hope that the demonstration of the production of vegetable starch will convince more workers as to the importance of this line of enquiry.

Since it is well known [see Harden, 1923] that the non-reducing carbohydrate of yeast is glycogen, it is of special interest that with *B. coli*, I find only starch, and I venture to suggest the possibility that some yeasts may synthesise starch also. This enquiry is being taken up. That there was an abundant formation of a non-reducing carbohydrate in the early stages of a bacterial fermentation was reported by myself in a paper from the Pasteur Institute [1918]. I did not know then that the substance was starch. It is probable however that there are several polysaccharides formed but they are not demonstrable by iodine, and cannot be so definitely characterised.

EXPERIMENTAL.

The essence of the experiment consists in removing samples during the early stages of a fermentation. The fermentation is brought about by mixing an emulsion of bacteria (which contains a certain amount of soluble nitrogenous matter), with a solution of glucose containing about 0.6 % of salts. Chalk is added to neutralise the acids which are produced by the fermentation. The fermentation is carried out in absence of air, but the bacteria employed have been grown previously aerobically on agar.

Arrangements for removing the samples. The technique previously described has been still further simplified. The fermentation is now carried out in a large evacuated flask, floating up-side-down in a large water tank kept at a constant temperature of 37°; the flask is enveloped in a net and secured by string to the sides of the tank. There is thus no strain and the flask however large has virtually no weight. The neck of the flask which is submerged, is provided with a rubber stopper through which two tubes pass, one into the liquid in the flask and the other into the gas space above. The rate of appearance of the fermentation gases in the evacuated flask may be measured by a mercury manometer. Since there are no joints above water and the flask is under reduced pressure there can be no question as to any gases being lost. In fact, it may be mentioned, that the idea in mind in adopting this arrangement was to investigate certain problems in connection with the gas production. These points will however not be discussed here.

In the experiment about to be described a ten litre flask was used and the volume of the fermenting liquid was three litres. Samples of about 500 cc. were removed at about six hourly intervals.

The samples are removed by connecting to the fermentation flask an evacuated bottle of the requisite size. Simultaneously with the removal of the liquid sample, a gas sample is removed, by the other tube which is connected to a mercury burette.

Experiment carried out February 20th-22nd, 1924. Organism of coliform type, in pure culture, with the following characteristics. Slightly motile, Gram negative, fermenting lactose, glucose, mannitol, with the production of acid and gas. Not fermenting dulcitol.

Composition of the fermenting fluid at the beginning of the experiment.

Glucose	...	...	...	...	60.4 g.
Bacteria	...	...	...	...	5.4 g.
Nitrogenous matter (mixed amino acids)					5.1 g.
Magnesium sulphate	...	...	...		3.0 g.
Potassium	„	...	...	...	15.0 g.
Tap water	...	...	...	...	3 litres
Chalk added	...	...	...	...	25 g.

Analysis of the carbohydrate present at various periods.

	Time in hours from the start	Glucose before hydrolysis g.	Glucose after hydrolysis g.	Synthetic carbohydrate present g.
1	0	60.4	60.4	—
2	9	52.5	52.5	nil
3	16	31.7	32.1	0.4
4	22	15.2	16.3	1.1
5	33	3.2	5.8	2.6
6	39	nil	1.8	1.8

The synthetic carbohydrate is rapidly hydrolysed by the bacterial enzymes, and the hydrolysis in many cases is found to occur in the presence of anti-septics; this point will be referred to elsewhere. Since however there is a continual decomposition of the complex carbohydrate it is clear that the amount present at any time is merely the excess of what is formed over what is decomposed so that the amount of this material actually built up by the bacteria is probably very much greater than appears from the table. Allowing for the portions removed in sampling we see that 3.2 g. of synthetic carbohydrate was present in the fermentation fluid, and it was found that 35.5 g. of glucose had been fermented. This is a yield of 9%. From the portion remaining after many analyses the starch was precipitated by the addition of alcohol and a weight of 0.60 g. was obtained, corresponding to 3.0 g. of starch in the total fermentation fluid. Starch was also present in the precipitates of calcium oxalate which are formed in the process of analysis, and mixed with the kieselguhr which is used for filtration, so that the amount obtained represents only a portion of the total.

The production of the synthetic carbohydrate measured by the optical rotation.

	Time (in hours)	Glucose % measured by the reduction method	Calculated rotation	Observed rotation (2 cm. tube)
1	9	1.68	1.78	1.82
2	16	1.33	1.49	1.72
3	22	0.91	0.96	1.54
4	33	0.46	0.48	0.93
5	39	0.32	0.33	0.69

The synthetic carbohydrate has clearly a high rotatory power. An actual measurement upon a sample of soluble starch isolated from the fermentation fluid gave the following result.

Observed in a length of 1 dm, a 0.712 % solution gave a rotation of 1.4° , $[\alpha]_D = 197^\circ$. This material was quite soluble in cold water, and had all the properties of soluble starch, it had however been obtained by prolonged boiling of the fermentation fluid which contained bacteria in suspension. I cannot say definitely whether any truly soluble starch exists preformed in the bacteria, there is none free in the fermentation fluid prior to boiling, but as it is so readily fermented it would doubtless be difficult to detect even if it escaped from the bacteria. The greatest part of the bacterial starch however is undoubtedly present in the substance of the bacteria and is liberated from them on prolonged boiling of the solution in which they are suspended.

The major part of the starch obtained in this experiment had already been degraded to a form of soluble starch or amylopectin before I was aware of its nature. This was the result of the prolonged boiling with oxalic acid solution which forms part of the routine analysis for the fermentation products. Some of the samples obtained possessed a definite reducing power, whilst others were quite devoid of any reducing power. The rotation of the latter was higher than that of those which, while giving a blue colour with iodine, also showed some reduction with copper solutions. A solution which showed no reduction was found to have an $[\alpha]_D$ of the order of 500° . This solution was obtained by adding hydrochloric acid to a bacterial sediment which contained chalk, and extracting the emulsion with ether, in a continuous apparatus; this treatment liberated the starch into the solution in a soluble form. A 0.01 % solution in a 20 cm. tube gave a rotation of 0.10° . The solution yielded a precipitate with alcohol, and after hydrolysis yielded phenylglucosazone of typical form.

CONCLUSIONS.

Bacteria synthesise starch abundantly from glucose. The synthetic side of bacterial metabolism is just as important as the decomposition which is such a characteristic feature. Probably a very large proportion of the various substances which are decomposed by bacterial fermentation are first synthesised into more complex materials within the bacterial protoplasm, and only subsequently undergo a decomposition into the end products which are commonly observed.

To study the formation of starch and similar substances by bacteria the fermentation must be interrupted at a very early stage. In the experiments described the synthetic period was passed in about 40 hours.

I wish to return my best thanks to my colleague W. M. Colles who has helped me in making many of the observations.

REFERENCES

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