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The fermentation of glucose by Fusarium lini.

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In a study of the biochemistry of *Fusarium lini*, the organism which causes flax wilt, its action on a nutrient solution containing glucose as the only source of carbon, has been investigated. Tochinai[1] in Japan has shown that *Fusarium lini* grows well on several of the common carbohydrates as a source of carbon. Of these he found inulin and glucose to give the best growth. In fermentation tests he found gas produced. Glucose produced gas in largest quantities, while galactose produced only traces. He considers this gas to be entirely CO_2. He suggests it is very probable that the wilt of the flax plant is due to the production of gas in the vascular system of the plant which interferes with transpiration.

In the present work a detailed study of the action of *Fusarium lini* on glucose has been made. The nutrient solution used had the following composition:

NH_4NO_3	1.00 g.
KH_2PO_4	.50 g.
$MgSO_4$	.25 g.
Glucose	20.00 g.
Distilled water to make	1,000 c.c.

The reaction of this medium was adjusted to the desired P_H by the addition of HCl or NaOH solutions. One hundred c.c. were placed in 300 c.c. Erlenmeyer flasks with 2-hole rubber stoppers fitted with cotton plugged glass tubes and rubber tubing with pinch clamps to prevent the escape of any gasses produced. These

[1] Tochinai, Y., *Annals of the Phytopathological Society of Japan*, 1920, i, 1; *Byochügai Zasshi*, 1921, viii, 2 (Japanese).

flasks were sterilized and inoculated with a definite volume of a spore suspension of *Fusarium lini* and incubated at 28 to 30 C.°. In the experiment here reported 15 flasks were used, the P_H being adjusted to 5.545, which is within the optimum reaction for this organism. These flasks were divided into sets of three each, and at intervals sets were removed and analyzed. Flask 1 was used for determining alcohol; flask 2 for mycelium, glucose, and lead precipitate; and flask 3 for mycelium and P_H. The CO_2 was determined on each flask at frequent intervals to avoid the development of pressure. The mycelium was separated from the medium by filtration onto a gooch. The carbon in the original culture, in the mycelium, and in the lead precipitate, was determined by wet combustion; alcohol was determined by aeration into concentrated H_2SO_4, oxidation with potassium dichromate to acetic acid, and distillation and titration of the latter; and glucose by the picramic acid method. Since in a previous experiment succinic acid had been identified as a product of the action of the organism on glucose, it was thought that lead acetate would precipitate any of this, hence the determination of the lead precipitate.

Chart I shows the results of the experiment. It is evident that over 90 per cent. of the glucose can be accounted for and that

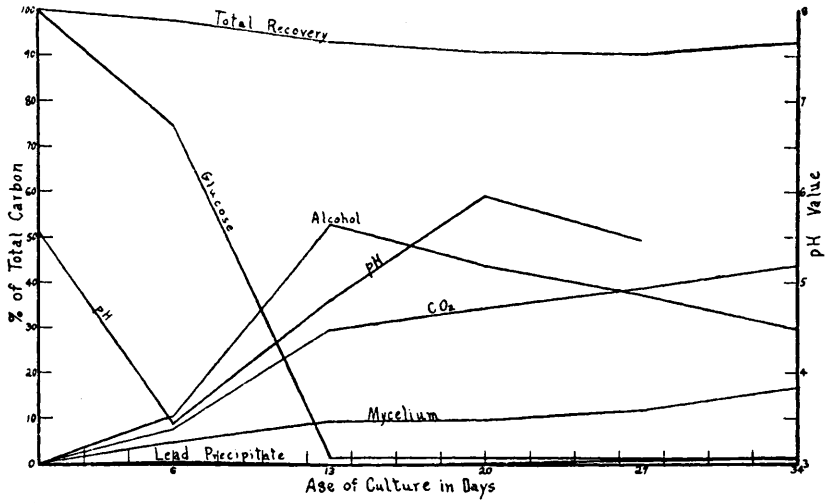

CHART I. Curves showing the percentage of carbon in the products of metabolism of *Fusarium lini* on glucose at different stages of growth. Change in P_H is also shown.

ethyl alcohol and CO_2 are the main products of the action of the organism on the glucose. This suggests an analogy to yeast fermentation. In the conventional equation for yeast fermentation, the carbon in the alcohol and in the CO_2 is in the ratio of 66.33; in the present data it is in the ratio of 62.35, which is a good agreement considering the fact that alcohol is consumed by *Fusarium lini,* as will be mentioned below. The other by-products of yeast fermentation, such as glycerol, acetone, and succinic acid, have not been identified in the present study, with the exception of one instance where succinic acid was found in small amounts. It is interesting to note that the quantity of alcohol increases until the glucose is gone, and that the fungus continues to grow at the expense of the alcohol, using it for growth and metabolism. The change in P_H of the medium is rather marked, and it appears to be due to a selective absorption of ions rather than to a production of organic acid. The lead precipitate is practically negligible and cannot account for any appreciable amount of succinic acid.

The same experiment was performed starting with media at a P_H of 3.685 and 8.960. The results were not essentially different from those here reported.

To prove more definitely that *Fusarium lini* can utilize ethyl alcohol as an only source of carbon, a series has been run in which increasing amounts of ethyl alcohol have been the only source of carbon. Good growth has been observed on cultures containing 4.04 per cent. of ethyl alcohol by volume. There was no growth on a culture containing 5.38 per cent. of alcohol. The best growth occurred where there was about 2 per cent. of alcohol.

We may conclude from the above study that *Fusarium lini* produces ethyl alcohol and carbon dioxide as the main by-products of metabolism when grown on glucose media, and that in the absence of glucose it can utilize the alcohol for metabolism and growth.