LXXV. BIOCHEMISTRY OF PLANT DISEASES.

XI. *FUSARIUM LINI* AND THE PYRUVIC ACID THEORY OF ALCOHOLIC FERMENTATION¹.

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PREVIOUS papers in this series [Anderson, 1924; White and Willaman, 1928] have brought out the facts that the principal products of metabolism of *Fusarium lini* Bolley, when grown on hexoses or pentoses, are carbon dioxide and ethyl alcohol, and that these two products, together with the mycelium produced, account for over 95 % of the sugar consumed. Certain comparisons with yeast fermentation are suggestive: the fungus and the yeast are alike in the ratio of CO₂ to alcohol produced on a hexose medium, but they differ in that ordinary yeast utilises the pentoses poorly if at all, that the fungus consumes the alcohol which it has produced, and that the rate of fermentation by the fungus is a matter of days instead of hours. Because, however, of the obvious similarity in end-products, it was thought desirable to examine the analogy still further. At the present time the pyruvic acid scheme of yeast fermentation is rather generally accepted. Since excellent reviews of this subject have lately appeared [Harden, 1923; Nord, 1926], it will be sufficient here simply to outline the supposed chain of events. The hexose molecule first is split into two three-carbon chains; methylglyoxal is the most reasonable of such three-carbon molecules that has been suggested, and it is the one usually adopted in the theory under discussion. Two molecules of this substance then undergo a "biological Cannizzaro" reaction, producing glycerol and pyruvic acid. The latter is decarboxylated to acetaldehyde which, with another molecule of methylglyoxal, then undergoes a mixed Cannizzaro reaction, with the formation of ethyl alcohol and of more pyruvic acid.

Although a great deal of work has been done to demonstrate this scheme in the case of yeast, only partial success has resulted. The proof would have to consist in showing that if any of the alleged intermediates are fed to the yeast the latter will utilise them for the production of alcohol and CO₂, and continue the course of events demanded by the scheme.

The present paper is a report of experiments in which the above intermediates were fed to *F. lini*, besides a number of other similar compounds.

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Methylglyoxal itself has proved to be too reactive a compound to be dealt with in cultures; therefore its hydroxy-derivative has been used instead. Glycerol has been shown by Tochinali [1921] to be readily used by the fungus, hence it was omitted here. Pyruvic acid and acetaldehyde are the other intermediates. Besides these, acetone, dihydroxyacetone, furfuraldehyde, oxalic and citric acids, and cinnamic aldehyde were used.

Growth on hydroxymethylglyoxal was immediate and fairly vigorous. Attempts were not made to measure the CO_2 and alcohol produced. Methylglyoxal itself was found by Mayer [1907] and by Wohl [1907] to be unfermentable by yeast. Buchner and Meisenheimer [1906] obtained the same results using both acetone-yeast and yeast juice. The products of fermentation would probably not be the same from the hydroxy-compound as from methylglyoxal, but such products have not as yet been looked for.

Growth on pyruvic acid was slower and less abundant than on the above, but production of CO_2 was very rapid. At the end of 12 days, 10 % of the original carbon was obtained in this form. Apparently the fungus can decarboxylate the acid very readily. Ethyl alcohol could not be detected. Since alcohol has been shown to be readily utilised by the fungus [Anderson, 1924], it is possible that, as fast as the pyruvic acid is decarboxylated to acetaldehyde, the latter is converted to alcohol and used by the fungus for growth. This view is supported by the fact that in an acetaldehyde culture growth readily takes place, with the production of only traces of CO_2 but of sufficient alcohol to be readily detected by the odour.

Acetone was tolerated in concentrations up to 10 %. Growth was immediate and rapid, but only small amounts of CO_2 were produced. No alcohol could be detected.

Dihydroxyacetone proved to be readily available for the fungus; a heavy mat of mycelium was produced, and CO_2 was formed in amount comparable to that on the pentoses.

We may now compare *F. lini* with yeast as regards the extent to which they have been demonstrated to fit into the pyruvic acid theory. Methylglyoxal has not been tried on the fungus; it is not utilised by yeast. Its hydroxy-derivative is not used by yeast [Nord, 1927], but it is by the fungus. Glycerol is utilised only by the fungus. Pyruvic acid is utilised by both, but much more readily for growth by *F. lini*¹. Acetaldehyde is available for both, and is detectable in the medium when both organisms are grown on hexoses [Letcher and Willaman, 1926]. Ethyl alcohol is produced by both, and is readily utilised by the fungus for growth, but not by the yeast.

Thus it has been demonstrated that each intermediate compound in the pyruvic acid theory of alcoholic fermentation may be utilised by *F. lini*, the methylglyoxal being represented by its hydroxy-derivative. Although ethyl

¹ Dr F. F. Nord was present when these experiments were conducted, and made some of the comparisons with yeast. The writers are also indebted to him for many suggestions during the course of this work, and for assistance in preparing the hydroxymethylglyoxal.

alcohol has not been found in all cultures, it is probably produced and then consumed by the fungus in preference to the original compound. Although these results do not constitute proof of the pyruvic acid theory for this fungus, nevertheless there seems to be a rather close analogy between the mechanism of alcohol fermentation in yeast and in *F. lini*.

EXPERIMENTAL.

Preparation of substances.

The *pyruvic acid* was purified by distilling it, at a pressure of 8 mm. at 55°, in a stream of CO₂.

The *cinnamic aldehyde* was distilled at a pressure of 10 mm. at 120°.

The *acetaldehyde* was not purified, but transferred to the sterile culture solution immediately after breaking the seal of the container.

The *hydroxymethylglyoxal* was prepared from dihydroxyacetone by the method of Evans and Waring [1926].

The *furfuraldehyde* was distilled at about 3 mm. pressure, the middle fraction of the distillate being used.

The acetone, dihydroxyacetone, and citric and oxalic acids were used as secured from the manufacturers.

Cultures.

The mineral nutrient solution used as a medium had the following composition: MgSO₄·7H₂O, 0.25 g.; KH₂PO₄, 0.25 g.; NH₄NO₃, 1.0 g.; water to make 1 litre.

100 cc. of this medium in a 300 cc. conical flask were sterilised, and then the organic compound was added. This was necessary because of the volatile nature of most of the substances.

Incubation was at 28°.

Alcohol and CO₂ were determined on some of the cultures. Since alcohol was found only in the acetaldehyde culture, and since the results here may have been vitiated by the volatility of the aldehyde, no alcohol data are presented.

The results are given in Tables I and II.

Table I. *Results of cultural studies on non-sugar media.*

Compound	No. of cultures	Range of concs. %		Conc. for best growth %	Time for noticeable growth days	Time for max. growth days	Type of final growth
Pyruvic acid	12	0.05 to	0.1	0.1	3	18	Fine mycelium
Acetaldehyde	16	0.01	0.2	0.1	2	9	" "
Acetone	16	0.01	10.0	10.0	0.5	21	Heavy clumps
Cinnamic aldehyde	6	0.25	1.0	No growth	No growth	No growth	
Citric acid	8	0.05	2.0	2.0	1	21	Heavy mat
Dihydroxyacetone	8	1.00	2.0	2.0	2	12	" "
Hydroxymethylglyoxal	6	0.01	0.1	—	1	12	Clumps of mycelium
Oxalic acid	4	0.05	0.1	—	3	10	Fine mycelium
Furfuraldehyde	4	0.10	0.5	0.1	1	—	" "

Table II. *Production of carbon dioxide by Fusarium lini on various non-sugar compounds.*

Compound	Conc. %	Carbon dioxide at the end of			
		3 days mg.	6 days mg.	9 days mg.	12 days mg.
Acetaldehyde	0.2	18.2	21.6	36.5	27.3
Acetone	10.0	12.9	21.6	30.8	22.8
Pyruvic acid	0.1	23.7	34.2	59.4	75.4
Dihydroxyacetone	2.0	20.9	32.9	29.6	59.4

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