STUDIES ON THE FOOD-RELATIONS OF *FUSARIUM LINI*.

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亞麻立枯病菌の營養に関する研究

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I. INTRODUCTION.

The food-relations of a fungus constitute one of the important factors which determine its parasitism and, on the other hand, the immunity or resistance of a host plant to a certain fungus. The investigation of the food-relations of a fungus is much more interesting from such a stand point.

The food demands of pure parasites are highly specialized; they seem to use the nascent or transitory substances which are produced by the assimilation or metabolism of their host plants. It may be possible that the so called obligate parasite is able to be cultured saprophytically by the close investigation of the character of such nascent or transitory forms of organic compounds.

Even in the case of facultative parasites, which are far less fastidious in their choice of nutriment, their development on artificial culture media containing certain substances is more or less affected by stereochemical differences of these substances.

It may be rational to consider that such a nutritive predisposition of a fungus must have importance in relation to parasitism and immunity.

Pasteur's success in artificial culture of bacteria marked an epoch in the science of mycology. Nägeli (1879) carried out an important investigation on the nutrition of fungi by carbon and nitrogen compounds. After him a considerable number of theses on the food relations of fungi and bacteria were published.

The present report includes only the results of the cultural studies of *Fusarium lini* which was carried out as a part of the investigations on this fungus.

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II. CARBON SOURCES.

I. Carbohydrates.

Carbohydrates are the most important source of carbon for fungi. They have the general formula $(C_6H_{12}O_6)_n - (H_2O)_{n-1}$, and the increase of the value of n marks the difference between mono-, di- and polysaccharides. The value of n also has important consequences to the availability of the carbohydrates in the nutrition of fungi. Moreover among a group which have the same value of n , some unknown differences of molecular constitution of each kind of carbohydrate have great meaning for the nutrition of fungi.

I have used a synthetic solution of the following formula as the standard nutritive solution.

Ammonium nitrate NH_4NO_3	1.00g
Potassium biphosphate KH_2PO_4	0.50g

Magnesium sulphate (crystal) MgSO_4	0.25g
Redistilled water H_2O	1000.00g

And I have used the following nine kinds of carbohydrates.

1. Glucose	$\text{CH}_2(\text{OH})(\text{CHOH})_4\text{CHO}$
2. Levulose	$\text{CH}_2(\text{OH})(\text{CHOH})_3\text{CO}\cdot\text{CH}_2$
3. Galactose	$\text{CH}_2(\text{OH})(\text{CHOH})_4\text{CHO}$
4. Sucrose	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
5. Maltose	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
6. Lactose	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
7. Soluble starch	$(\text{C}_6\text{H}_{10}\text{O}_5)_n$
8. Inulin	$(\text{C}_6\text{H}_{10}\text{O}_5)_n$
9. Arabin	$(\text{C}_6\text{H}_{10}\text{O}_5) + \text{H}_2\text{O}$

Solutions containing 2% of each of these carbohydrates were prepared by adding 4 grams of the carbohydrates to 200cc. of standard solution. Fifty cubic centimeters portions of this solution were placed in each of four Erlenmeyer's flasks of 200cc. capacity. They were autoclaved about 30 minutes three times with 24 hours' intervals, and inoculated with a bit of mycelium, and incubated at 28°C. for a fortnight.

After two days, growth was visible in every flask.

After ten days, in the flasks containing inulin showed the best development of the fungus, and a vigorous growth of the white aerial mycelium covered all over the surface of the nutrient solution. In the flasks containing glucose and soluble starch the fungus developed also very well. In the flasks containing sucrose the developments of the fungus were less vigorous. In the flasks containing levulose and galactose the fungus developed much more less vigorously. The solutions containing arabin and maltose showed far less developments of the fungus. The lactose solution was least favorable to the development of the fungus; a scanty thin mycelial layer spreading over the surface of the solution.

After a fortnight the colonies which had developed in each solution were strained on pieces of filter paper of known weights, and washed with distilled water several times. Then after the greater part of the water had been evaporated in a vacuum desiccator they were placed in a calcium chloride desiccator for a long time, and weighed. The average weights of the mycelia were as follows.

Kinds of the carbohydrate	Average weights	Apparent growth of aerial mycelium
1. Glucose	0.274g	very good
2. Levulose	0.084	moderate
3. Galactose	0.072	moderate
4. Sucrose	0.044	good
5. Maltose	0.141	moderate
6. Lactose	0.039	a little
7. Soluble starch	0.125	good
8. Inulin	0.299	very good
9. Arabin	0.147	moderate

From these results it is evident that inulin and glucose are the carbohydrates best suited for the nutrition of this fungus and lactose is the worst of all. The weight number for the sucrose cultures is rather small. In the disaccharides maltose is conspicuously good as glucose is among the monosaccharides. On the whole the polysaccharides seem generally to be favorable carbon sources for this fungus, such obvious differences of developments of the fungus for the kinds of carbohydrates are probably related to the kinds of enzymes produced by the fungus.

II. Organic Acids.

Standard solution, preparation of culture and cultural conditions were all the same as in the preceding experiment.

The following 6 kinds of organic acids were used for the source of carbon.

1. Malic acid

2. Succinic acid

3. Maleic acid

4. Fumaric acid

5. Racemic acid (d-tartaric acid + l-tartaric acid)

6. Citric acid

In these acids, malic acid is formed by replacing the H of succinic acid with OH; maleic acid and fumaric acid are stereoisomers, and they differ from each other only the position of H and COOH. It is interesting to know that such a difference of chemical constitution should have any effect on the nutrition of the fungus.

Racemic acid is an equivalent mixture of d-tartaric acid and l-tartaric acid, and is optically inactive by external compensation. There is one more kind of tartaric acid named mezo-tartaric acid which has the following molecular structure,

, and is optically inactive by internal compensation.

The optically inactive nature of racemic acid and mezo-tartaric acid are wholly different, in that the former is inactive for a rather mathematical reason and the latter by chemical molecular construction.

In the racemic acid culture, if the fungus assimilates only the one kind of tartaric acid i.e., d- or l-, the cultural solution must become optically active. Then the polarimetry of the cultural solution after a certain development of the fungus, may show the affinity of the fungus for these two kinds of tartaric acids either positively or negatively.

One half per cent of these acids checked the development of the fungus, except succinic acid. A concentration of only 0.1% was then tried.

The results of 14 days culture is as follows

1. Control (no acid, 0.1% sucrose is used for carbon source)	good	++++
2. Succinic acid	good	+++
3. Malic acid	fair	++++
4. Citric acid	a little	+++
5. Fumaric acid	„	+++
6. Maleic acid	poor	++
7. Racemic acid	slight	+

In these results fumaric acid cultures were better than those of its isomer maleic acid. It is interesting that the molecular construction of fumaric acid

In racemic acid cultures I have examined the optical activity of the nutrient solution at the end of the experiment. This solution has shown optically active character as $L-2^\circ$ in a tube of 200mm. length. From this result it may be concluded that *Fusarium lini* assimilates d-tartaric acid better than l-tartaric acid.

But the chemical or biological reason of such a difference of nutritive affinities of some compounds for a fungus is yet remained as a vital question now-a-day.

3. Higher Alcohols and Phenol Derivatives.

I have selected glycerine and mannite for higher alcohols, and vanillin, thymol and α -naphthol for phenol derivatives.

Their chemical formulae are as follows:—

Glyceline	$\text{CH}_2(\text{OH})\text{CH}(\text{OH})\text{CH}_2\text{OH}$	
Mannite	$\text{CH}_2(\text{OH})\text{CH}(\text{OH})\text{CH}(\text{OH})\text{CH}(\text{OH})\text{CH}(\text{OH})\text{CH}_2\text{OH}$	
Vanillin	$\text{C}_6\text{H}_5\text{CHO}-\text{OCH}_3\text{OH}$	$ \begin{array}{c} \text{CHO} \\ \diagdown \quad \diagup \\ \text{C}_6\text{H}_4 \\ \diagup \quad \diagdown \\ \text{OCH}_3 \\ \text{OH} \end{array} $
Thymol	$\text{C}_6\text{H}_5\text{CH}_2\text{OH}-\text{CH}(\text{CH}_3)_2$	$ \begin{array}{c} \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{C}_6\text{H}_4 \\ \diagup \quad \diagdown \\ \text{OH} \\ \text{CH}(\text{CH}_3)_2 \end{array} $

α -naphthol $C_{10}H_7OH$

The preparation of cultures was just the same as in the two preceding experiments, each solution containing 2% of one of the above-named compounds. All experiments were in triplicate.

The results of cultures after a fortnight were as follows:—

Glycerine	a little, dry weight of mycelium averaged 0.024g.
Mannite	very good, dry weight of mycelium averaged 0.24g.
Vanillin	none
Thymol	none
α -naphthol	none

In these results, mannite was a good carbon source for this fungus, and glycerine was able to be assimilated by this fungus but poorly. But these kinds of phenol derivatives were all poisonous for this fungus, utterly preventing its development.

III. NITROGEN SOURCES.

1. Organic and Inorganic Nitrogen Compounds.

Fungi never assimilate carbon dioxide; organic carbon compounds are always necessary for their development. But a fungus can assimilate nitrogen from either inorganic nitrogensalts or from organic nitrogen compounds. Moreover some species of fungi and bacteria fix free nitrogen from air.

I have carried out the following experiment to determine what nitrogen compounds are the most favorable for this fungus.

I have used the following kinds of nitrogen compounds for the nitrogen source of this experiment.

Inorganic compounds.

1. Ammonium phosphate	$(NH_4)_2PO_4$
2. Ammonium sulphate	$(NH_4)_2SO_4$
3. Sodium nitrate	$NaNO_3$
4. Potassium nitrite	KNO_2

Organic compounds

5. Peptone

6. Urea

A synthetic nutrient solution of the following formula was used as the standard medium.

Potassium biphosphate	KH_2PO_4	0.50 g.
Magnesium sulphate (Crystal)	MgSO_4	0.25 g.
Cane sugar	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$	20.00 g.
Redistilled water	H_2O	1000.00 cc.

From the standard solution the nutrient solutions were prepared, each containing 2% of one of the nitrogen compounds mentioned above. Preparation of culture was the same as in the preceding cases. All the kinds of cultures were in triplicate. The results of cultures after a fortnight were as follows:—

Kinds of N-compound	Apparent growth of aerial mycelium	Average dry weight of colonies
$(\text{NH}_4)_2\text{PO}_4$	very good	0.194 g.
$(\text{NH}_4)_2\text{SO}_4$	a little	0.038 g.
NaNO_3	good	0.198 g.
KNO_3	slight	0.012 g.
Peptone	very good	0.448 g.
Urea $\text{NH}_2\text{CO}\cdot\text{NH}_2$	good	0.251 g.

These results show that the organic nitrogen compounds are far better nitrogen source for this fungus than inorganic nitrogen compounds. Especially peptone is the most nutritious as in the cases of many other fungi.

In the inorganic compounds, there is no difference of nutritive value between nitrogen in ammonia form and in nitrate form. The suitability of inorganic nitrogen compounds as the nitrogen source for this fungus depends rather on the properties of the compound than on the form of the nitrogen; but nitrite (NO_2) is not a good nitrogen source for this fungus.

3. Amides.

The amide group (NH_2) has an important nutritive role for the life of hetero-

trophic plant as the nitrogen source. In the bodies of autotrophic plants nitrogen appears often as amides or amino-acids in the processes of assimilation or metabolism of nitrogen.

I have carried out an experiment on the food relation of amide compounds for this fungus.

Standard nutrient solution and other cultural preparations were the same as in the preceding experiment.

For the amides the following compounds were used, to the extent of one per cent of the standard nutrient solution.

1. Acetamide	$\text{CH}_3\cdot\text{CO}\cdot\text{NH}_2$
2. Succinamide	$\begin{array}{c} \text{CH}_3\cdot\text{CO}\cdot\text{NH}_2 \\ \\ \text{CH}_2\cdot\text{CO}\cdot\text{NH}_2 \end{array}$
3. Dicyandiamide	$\text{NH}\cdot\text{C} < \begin{array}{c} \text{NH}_2 \\ \text{NH}\cdot\text{CN} \end{array}$
4. Asparagine	$\begin{array}{c} \text{NH}_2\cdot\text{CH}\cdot\text{COOH} \\ \\ \text{CH}_2\cdot\text{CO}\cdot\text{NH}_2 \end{array}$

The cultures were all in triplicate.

The developments of the fungus after the culture of a fortnight were as follows :—

Kinds of amide	Apparent growth of aerial mycelium	Average dry weight of the colonies
Acetamide	good	0.173 g.
Succinamide	moderate	0.143 g.
Dicyandiamide	moderate	0.156 g.
Asparagine	very good	0.288 g.

In these results, it is evident that the amide compounds are generally favorable nutritive substance for this fungus. In this experiment, considering the rather low percentage of amide compounds, on the whole, the development of the fungus was good. Asparagine is an especially favorable nutriment for this fungus.

IV. SUMMARY.

1. Inulin and glucose are the most suitable carbohydrates for the carbon source for this fungus, and lactose is the worst of all.

2. Polysaccharides are generally favorable carbon sources for this fungus.
3. Organic acids as carbon sources are unfavorable to this fungus, and it can develop only in low percentages as 0.1%.
4. Maleic acid and fumaric acid have in some respect an unequal nutritive value to this fungus notwithstanding they are different only in stereochemical construction of molecule.
5. In the nutrient solution which contains racemic acid as the carbon source, this fungus seems to assimilate d-tartaric acid better than l-tartaric acid.
6. Of these acids succinic acid is the most favorable carbon source.
7. Mannite is a suitable carbon source.
8. Phenol derivatives check the development of the fungus in many cases.
9. Organic nitrogen compounds are better than inorganic nitrogen salts as the nitrogen source for this fungus.
10. Ammonia nitrogen and nitrate nitrogen are equal in their nutritive value to this fungus, but nitrite is unsuitable.
11. Amides are generally good nitrogen source for this fungus, and asparagine is the best of all.

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摘 要

病原菌の營養の關係は、菌の寄生性及び寄主植物の免疫性或は抵抗性に影響する點に於て、特に興味深き問題なり。余は亞麻立枯病菌の生理的研究の一部として、炭素源及び窒素源に関する試験を行ひ、次の如き結果を得たり。

本菌の炭素源としては各種の炭水化物、有機酸、高級酒精及びフェノール化合物を以て試験を行ひたり。

炭水化物にありてはイヌリン及葡萄糖は最適當にして、乳糖は最不適當なり。一般に多糖類は良好なる發育をなさしめたり。

有機酸は本菌に對する炭素源としては不適當にして、菌の發育貧弱なりき。有機酸の分子構造の差が、其の營養價に比較的明かなる差違を生ぜしむる事は著しき事實にして、例へばマレイン酸とフマル酸とは同一の分子式を有し、僅かに其の構造式に於て異なるのみなれども、菌の發育はフマル酸に於てより良好なりき。又ラセミ酸を炭素源として與へたる場合に、本菌は右轉性酒石酸を多く消費し、培養液に左轉性を現ぜしめたり。有機酸のうちにては琥珀酸は比較的適當なる炭素源なり。

高級酒精のマンニツトは本菌に對し良好なる炭素源にして、フェノール誘導體は一般に有害作用を呈す。

窒素源としては有機窒素化合物は無機含窒素鹽類よりも適當にして、安母尼體の窒素と硝酸體窒素との間には營養上の差なく、亞硝酸體の窒素は不適當なり。

アミド類は一般に、本菌の窒素源として適當にして、就中アスパラギンは最も良好なり。