EFFECT OF VARIOUS CARBON AND NITROGEN SOURCES AND CONCENTRATIONS ON THE GROWTH OF FUSARIUM DIMERUM PENZIG

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The effect of various amino acids and carbohydrates as sources of nitrogen and carbon respectively on *Fusarium dimerum* Penzig has been studied. Different concentrations of nitrogen and carbon were used. Neutral, acidic and basic amino acids and mono-, di-, tri-and polysaccharides were used. It was found that glycine gave the most profuse growth of the fungus at all concentrations and maximum growth was obtained at 0.1%. The growth thus was directly proportional to the concentration of nitrogen. Best growth in glycine may be either due to its simple structure, making the nitrogen easily available for growth or due to possible specificity of the fungus for glycine as compared to other nitrogen sources.

In case of carbohydrates, trisaccharide raffinose and polysaccharide, inulin gave better results than other carbohydrates. This behaviour may be due to two factors. As the results were taken after a period of 10 days, it is quite possible that in the initial stages of growth i.e. after 2 or 3 days, the growth may be more in monosaccharides than in tri-and polysaccharide. However, as the tri-and polysaccharide were hydrolysed by the fungus in due course and the sugar became more easily available for growth, the amount of growth increased rapidly. Secondly the possible specificity of *F. dimerum* for trisaccharide raffinose and polysaccharide inulin may also be responsible for this behavior.

Introduction

Availability of carbon and nitrogen sources are amongst the prime conditions besides suitable temperature, pH, humidity and environment for the growth and development of any type of microorganisms. Temperature affects growth, spore germination, reproduction and indeed all activities of the organisms but this again is valid under specified conditions of time, medium, aeration, moisture content, pH and the method of growth measurement.

Though numerous surveys of nitrogen nutrition have been made but without much conception of the physiological problems involved. Not all nitrogen sources are equally suitable for all fungi. Fungi may be specific in the nitrogen source they utilize.[1] Steinberg[2] made an extensive study of the growth of *Aspergillus niger* on 22 amino acids. Of these, seven acids, namely, alanine, arginine, aspartic acid, glycine, glutamic acid, proline and hydroxyproline, were excellent sources of nitrogen for *A. niger*. This is due to specificity of response to certain amino acids by a certain fungus.

Amino acids are assimilated at varying rates from a complex medium and they exist as free acids in the mycelium.[3] Experiments of this type have shown that a given amino acid allows good growth of one organism and only little of another, but still we are unable to conclude whether this selective behaviour of the two organisms involved is due to permeability, enzymatic capacities or merely due to secondary problems.

Most investigators agree that glycine, aspartic acid, asparagine, and glutamic acid are most likely to support good growth of microorganisms.[3] Though leucine is generally not an adequate source of nitrogen,[4] it is one of the most easily utilizable amino acid for *Trichophyton persicolor*.[5]

Regarding the utilization of carbon much valuable work has been done as compared to the utilization of nitrogen. Carbon is one of the most important elements used in fungal metabolism. It occupies a unique position in the metabolic pathway. It is also an integral constituent of fungal structure. The synthesis of complex compounds like proteins and nucleic acids in the cell and the accumulation of chitin and cellulose in their wall strongly confirms the necessity of carbon. Carbon also liberates a considerable amount of energy by oxidation which is one of the essential processes carried out into the organisms.

The multidimensional studies on carbon nutrition, its presence or absence and corresponding changes in the growth of the organism have opened a new avenue in fungal physiology. Though tremendous amount of work has been done on this subject, yet the method, technique and use of different carbon sources in same or in different media, has been a major controversy among fungus physiologists.

More is known about carbohydrates as carbon source which enables us to get an insight into the manner of carbon assimilation. All fungi are unable to utilize exactly the same source of carbon. The utilization depends on the configuration of carbon compound and the ability of a certain fungus to utilize a specific source of carbon.[1]

Holzapfel[6] worked on *Fusarium sambucinum* under various cultural conditions to calculate the relationship between the amount of fungus metabolic product and the amount of carbon used. This fungus utilized sucrose and fructose more efficiently than glucose. Steinberg,[2] Peterson *et al*,[7] White and Willaman[8] and Fries[9] worked on the utilization of various carbohydrates by fungi. Nord and Mull,[10] considered that species of fungi dissimilate carbohydrate by oxidation, by splitting carbon chain and by phosphorylation mechanisms. These three mechanisms depend on the fungus involved and various environmental conditions.

Since no work has been done on the nutritional requirements of *Fusarium dimerum*, the present work was undertaken for studying the effect of various sources and concentrations of nitrogen and carbon on the growth of this particular organisms, and for establishing the specificity of this organism towards them.

Materials and Methods

The effect of various amino acids and carbohydrates as a source of organic nitrogen and carbon respectively on *Fusarium dimerum* was determined in Czapek's Liquid medium. This liquid medium is apparently most suited for fungal growth. Constituents of the medium were:–sodium nitrate 2.0 g, potassium dibasic phosphate 1.0 g, potassium chloride 0.50 g, magnesium sulphate 0.50 g, ferrous sulphate 0.01 g, sucorse 30.0 g, distilled water 1000.0 ml., pH 6.2.

To this basal Czapek's medium various amino acids were added separately instead of $NaNo_3$ which is the normal source of nitrogen in this medium. The concentration of nitrogen was kept the same irrespective of which amino acid was used. In case of carbohydrates, sucrose from the original medium was replaced by other carbohydrates which were used as sources of carbon. The pH in all cases was adjusted to the same value. The amino acids and carbohydrates used were :

Amino Acids

Neutral :	glycine, serine, alanine, methionine, valine and phenylalanine.
Acidic :	aspartic acid and glutamic acid.
Basic :	lysine and arginine.

Carbohydraes

Monosaccharides:	fructose, mannose, galactose and glucose.
Disaccharides:	maltose and lactose.
Trisaccharide:	raffinose.
Polysaccharides:	inulin and starch.

Five concentrations of nitrogen, 0.01, 0.02, 0.03, 0.04 and 0.1% were taken and three flasks for each concentration were used. These concentrations were used for all the amino acids. Flasks containing the medium and the amino acids were autoclaved at 15 lb pressure for 15 minutes (121° C), allowed to cool and then inoculated with 4 mm discs cut from the growing edges of 4-day old culture of *F. dimerum* which was growing on Czapek's solid medium. The flasks were incubated at 28°C. After 10 days the cultures were filtered (on already weighed filter papers), dried at 45°C and then weighed. The difference between the original and final weight (with mycelium) of filter paper gave the weight of mycelium at different concentrations. The dry weight of mycelium was plotted against nitrogen concentration.

Weighed amounts of sugars mentioned earlier were added to Czapek's medium in five triplicate sets of flasks to obtain a concentration of 0.3, 1.0, 2.0, 3.0 and 4.0%. Further procedure was the same as for nitrogen.

Results

The effect of different concentrations of nitrogen and carbon from different sources on *F. dimerum* has been measured by the amount of growth obtained after an incubation period of 10 days at 28°C. Among 10 amino acids used, best growth was obtained on glycine and serine at concentrations of 0.1% of nitrogen (Fig. 1) 822 and 782 mg, respectively which subsequently was the maximum concentration used. The other amino acids alanine, arginine, methionine, lysine, valine, phenylalanine, glutamic acid and aspartic acid produced growth in a descending order which was 701, 619, 550, 546, 531, 488, 395 and 348, respectively. Thus the growth of *F. dimerum* is directly proportional to the concentration of nitrogen. As the concentration of nitrogen increases from 0.01, 0.02, 0.03, 0.04 to 0.1% the growth increases in all cases (Figs. 2, 3, 4).

In case of carbohydrates, the trisaccharide raffinose and polysaccharide inulin has given best growth (3012 and 2811 g, respectively) (Fig. 5). The other polysaccharide starch has given comparatively less growth (1104 mg) (Fig. 6, 7). Next to polysaccharides were the monosaccharides fructose and mannose which produced equal amount of growth (2588 and 2578 mg) while the other two monosaccharides glucose and galactose yielded less growth (1810 and 1808 mg respectively) (Fig. 8).

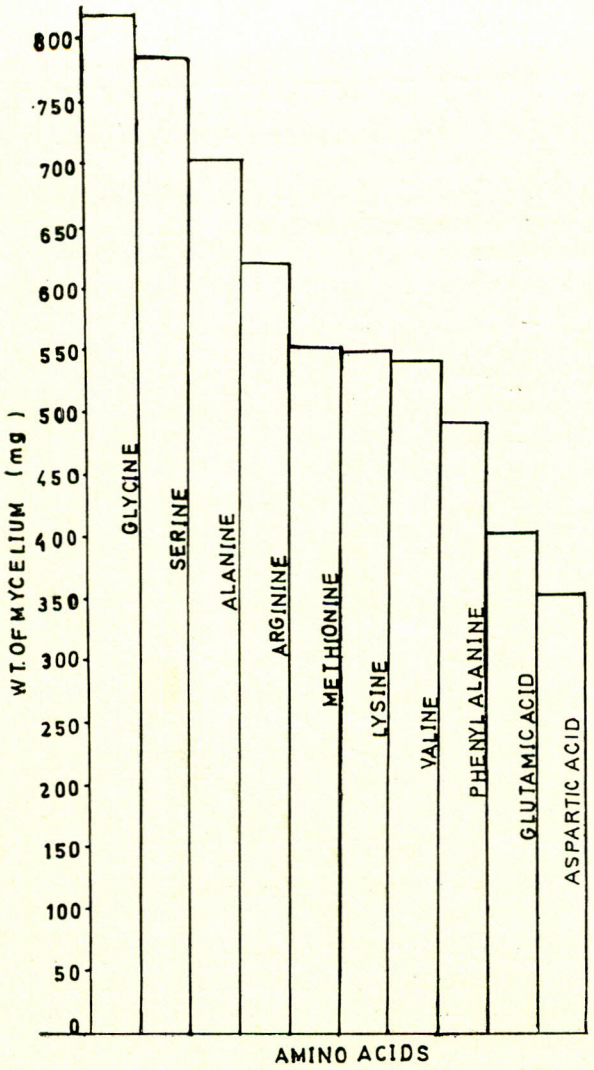


Fig. 1.—Effect of ten different amino acids on the growth of *Fusarium dimerum* at a nitrogen concentration of 0.1%, maximum growth in glycine.

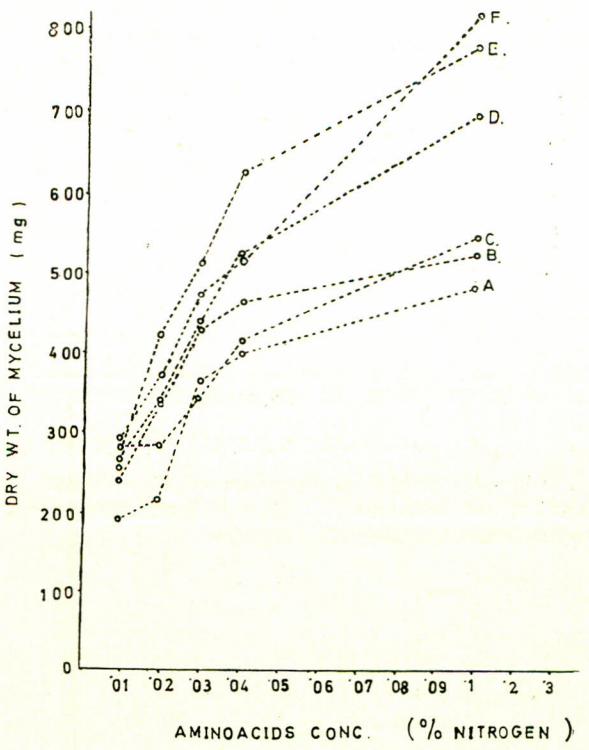


Fig. 2.—Dry weight of mycelium of *F. dimerum* obtained in six neutral amino acids at different nitrogen concentrations. A, Phenylalanine; B. Valine; C, Methionine; D, Alanine; E, Serine; F, Glycine.

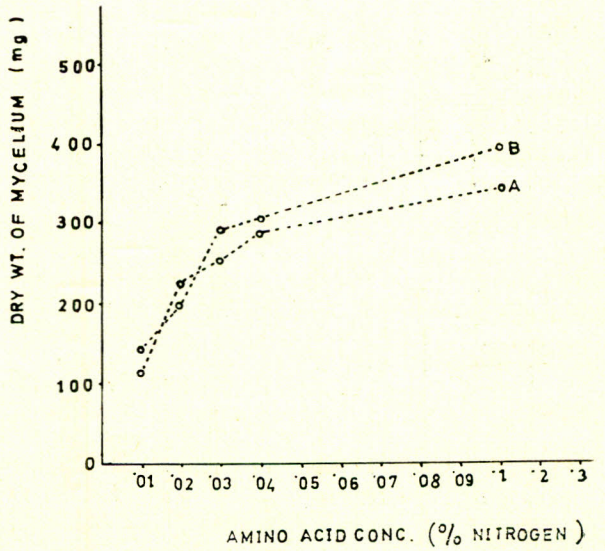


Fig. 3.—Dry weight of mycelium of *F. dimerum* obtained in two acidic amino acids at different concentrations of nitrogen. A, Aspartic acid; B, Glutamic acid.

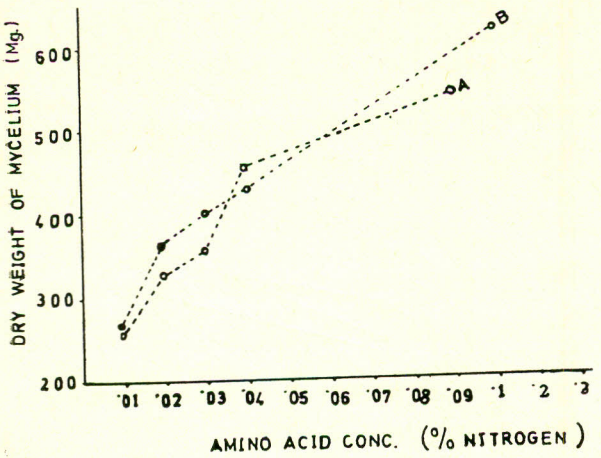
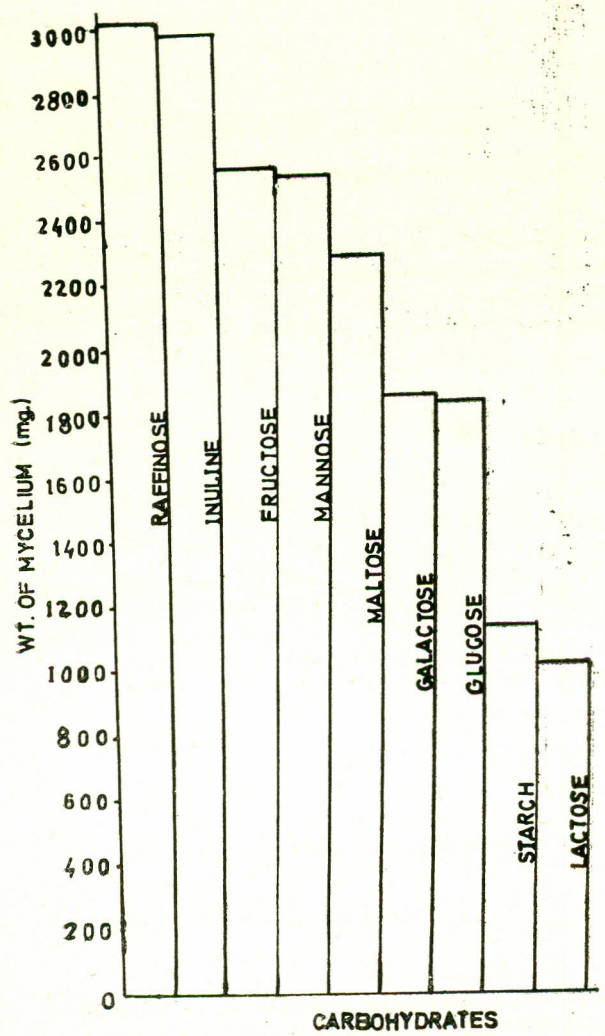


Fig 4.--Dry weight of mycelium of *F. dimerum* obtained in two basic amino acids at different nitrogen concentrations. A, Lysine; B, Arginine.



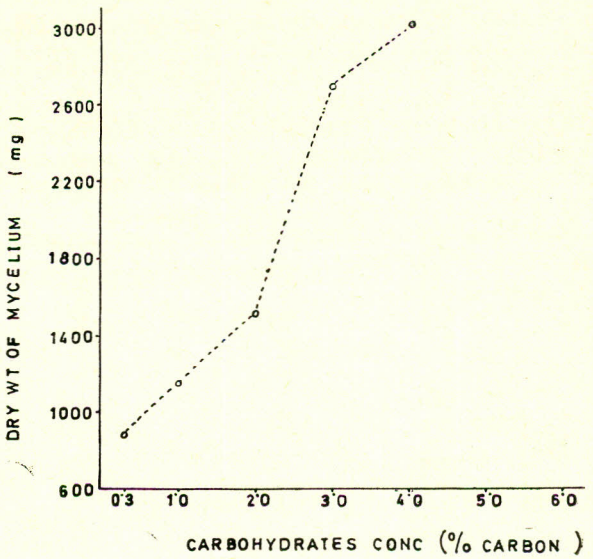


Fig. 6.—Dry weight of mycelium of *F. dimerum* obtained in trisaccharide Raffinose at different carbon concentrations.

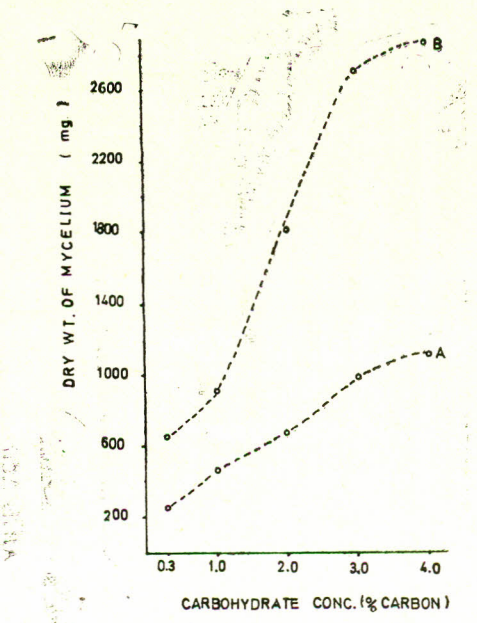


Fig. 7.—Dry weight of *F. dimerum's* mycelium obtained in polysaccharides at different concentrations. A, Starch; B, Inulin.

Fig. 5.—Effect of nine different sugars on the growth of *F. dimerum* at a carbon concentration of 4%. Observe maximum growth in raffinose.

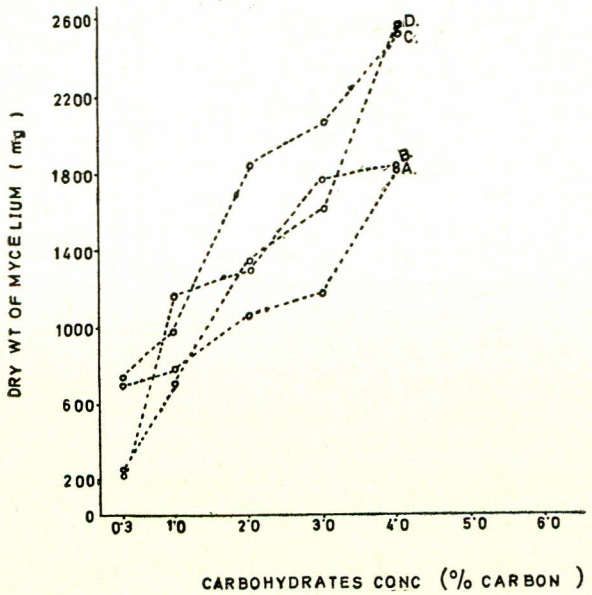


Fig. 8.—Dry wegith of *F. dimerum's* mycelium obtained in monosaccharides at different carbon concentrations. A, Galactose; B, Glucose; C, Mannose; D, Furctose.

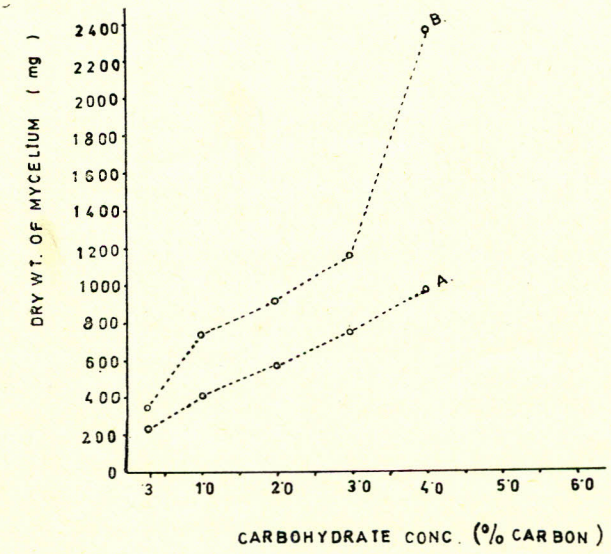


Fig. 9.—Dry weight of mycelium of *F. dimerum* obtained in disaccarides at different carbon concentrations. A, Lactose; B, Maltose.

The disaccharide lactose was least helpful in promoting the growth of the fungus (987 mg) while the other disaccharide maltose was a better source of carbon than the two monosaccharides galactose and glucose in producing a growth of 2291 mg. (Figs. 8, 9).

Discussion

In the present studies ten amino acids belonging to neutral, acidic and basic groups were used as sources of nitrogen for the growth of *F. dimerum*. Four amino acids, glycine, serine, alanine and lysine were found to be excellent for the growth of this organism. Though the nitrogen percentage of each amino acid used and the environmental conditions such as pH, temperature and incubation period were the same, the results obtained were different. Apparently *F. dimerum* is selective in utilizing different nitrogenous compounds as source of nitrogen. This capacity differs in different strains of the same organism.

Lilly and leonian, [11] investigated the effect of nitrogen on the growth of 10 strains of *Saccharomyces cerevisiae*. Their analysis showed that different amino acids vary in their effectiveness and that different strains of the same oragnism behave differently to the same source of nitrogen. This demonstrated that utilization of amino acids vary from strain to strain in spite of similar environmental conditions. One amino acid may be an excellent source of nitrogen for the growth and metabolic activity of one fungus but may be of no value to another.

The present work on *F. dimerum* suggests that glycine is one of the best sources of nitrogen for growth and other activities of the fungus. However Rayan's [12] work shows that glycine is harmful for *Neurespora crassa* so much so that its spore germination is inhibited by glycine.

Studies on carbon nutrition showed that of the nine different carbohydrates representing mono-, di-,tri- and polysaccharides, best results were obtained on trisaccharide raffinose and polysaccharide inulin and then on the monosaccharides fructose and mannose. The fact that raffinose and inulin support better growth of *F. dimerum* than fructose and mannose is somewhat unusual because the latter sugars are more readily available for utilization by the fungus than the former. This behaviour may be due to two factors. As the results were taken after a period of 10 days, it was quite possible that in the initial stages of growth i.e. after 2 or 3 days the growth in monosaccharides may be more than that in the trisaccharide and polysaccharide. However, as the tri and polysaccharides were hydrolysed by the

fungus in due course of time and the sugar became more easily available for growth, the amount of growth increased rapidly. Secondly the possible specificity of *F. dimerum* for raffinose and inulin may also be playing a major role. Therefore, if results were taken after an incubation period of 10 days the above two factors combined together may produce more mycelial growth in trisaccharide and polysaccharide than in monosaccharides. However, more needs to be known about this behaviour of *Fusarium dimerum*.

Different workers have shown the specificity of microorganisms for different sugars. Herrick[13] observed that *Stereum gausapatum* utilized xylose better than arabinose. Tamiya[14] found that *Aspergillus oryzae* utilized arabinose better than xylose. Nord and Vitticci [15] noted that *Lentinus lepideus* utilizes xylose only.

Work on the nutrition of fungi with a view to study the metabolic products at different concentrations of nitrogen and carbon from different sources are continuing and interesting results are expected.

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