Effect of nitrogen sources on the growth of Pythium aphanidermatum (Edson) Fitz

By Rajendra K. Grover and J. S. Sidhu

Department of Botany and Plant Pathology, Punjab Agricultural University, Ludhiana (Pb.), India.

The damping-off of seedlings and root-rot of various crop plants and vegetables are caused by *Pythium aphanidermatum* (Edson) Fitz. The disease is both soil borne and seed borne and is world wide in distribution. Information regarding the nutritional studies of *Pythium* species is very scanty and has been reviewed by Kumar and Grover (1966). The present paper deals with the effect of various nitrogen sources on the growth of *P. aphanidermatum* .

Materials and Methods

The culture of *P. aphanidermatum* was the same as used by Kumar and Grover (1966). Stock cultures were maintained on Potato dextrose agar medium. The result of preliminary experiments with different basal media and nitrogen sources showed that best growth was obtained after 8 days on a medium containing sucrose 30 g; $NaNO_3$ 2 g; KH_2PO_4 1 g; $MgSO_4 . 7 H_2O$ 0.5 g; KCl 0.5 g; $FeSO_4$ 0.01 g; and distilled water to make 1 liter. The nitrogen compounds, organic or inorganic, were added to the basal medium in place of $NaNO_3$ in amounts calculated to give equivalent concentrations of nitrogen. The composition of casein hydrolysate given by Horrow (1946) was used as a guide in selecting nitrogen concentrations. Amino acids were of reagent grade (B. D. H., London) and casein hydrolysate was supplied by Oxo Ltd., London. The inorganic and other chemicals used for different basal media were of analytical grade supplied by B. D. H., London, or E. Merck & Co., Germany. The pH of all the media was adjusted to 5.5 with 0.1 M HCl or NaOH, before sterilization. All culture vessels used were of Pyrex glass.

The basal medium was made twice the final concentration and 25 ml. aliquots dispensed in each of 250 ml Erlenmeyer flasks. To this calculated amounts of respective nitrogen compounds from stock solutions were added and the total volume of solution per flask was made upto 50 ml. with distilled water. The medium was then sterilized for 20 minutes at 15 lb. pressure. Since the fungus did not sporulate, the inocula-

tions were made from 4 days old mycelial cultures growing on 2% plain agar medium. The inoculum for each flask consisted of 4 mycelial disks (5 mm. diameter) cut from the peripheral end of the cultures with sterile cork borer so that actively growing regions of same physiological growth could be obtained. Unless otherwise stated, the cultures were incubated for 7 days at 26 ± 1° C, after which the mycelium from replicate flasks of each treatment were combined, filtered, washed, dried in a hot air oven and finally weighed. The dry weight of the mycelium was taken as a measure of the growth. All the cultures were maintained in triplicate and each experiment was repeated at least twice.

Results

Effect of different Nitrogen sources: Five inorganic nitrogen compounds along with urea and casein hydrolysate were incorporated in the basal medium as the sole nitrogen source in order to see their effect on growth (Table 1).

Table 1. Mycelial dry weight of *Pythium aphanidermatum* on media containing different nitrogen sources.

Nitrogen source	Nitrogen Concentration µg/ml.	Dry weight mg.	Final pH
Sodium nitrate	140	186	6.6
Potassium nitrate	140	180	6.5
Potassium nitrite	140	0	5.5
Ammonium chloride	140	66	4.8
Ammonium nitrate	140	93	5.2
Urea	100	136	4.4
Casein hydrolysate	100	238	4.8

A perusal of Table 1 indicates that organic nitrogen compounds were better sources of nitrogen than nitrate nitrogen or ammonium nitrogen. Nitrite was not utilized by the organism. A downward trend in final pH was observed when amonium or organic nitrogen compounds were used; wheares in the presence of inorganic nitrates the final pH increased.

To determine the effect of nitrogen concentration on the growth of *P. aphanidermatum,* the organism was grown on different concentrations of potassium nitrate, ammonium nitrate and casein hydrolysate. The results are presented in Table 2.

Table 2. Mycelial dry weight of *Pythium aphanidermatum* when grown on media containing different nitrogen concentrations of potassium nitrate, ammonium nitrate or casein hydrolysate.

Nitrogen source	Nitrogen Concentration µg/ml.	Dry weight mg.	Final pH
Potassium nitrate	70	242	6.6
	140	184	6.4
	280	135	6.2
	350	79	5.8
	420	43	5.6
Ammonium nitrate	70	101	5.4
	140	91	5.2
	280	77	5.1
	350	65	5.0
	420	38	5.0
Casein hydrolysate	50	215	5.2
	100	240	4.8
	250	258	4.6
	500	456	4.5

It may be seen from Table 2 that potassium nitrate and ammonium nitrate when added to the basal medium in different concentrations yielded maximum growth at low concentrations of nitrogen, while with the increase in nitrogen concentration of casein hydrolysate an increase in growth was observed. It became clear that *P. aphanidermatum* had a great specifity of nitrogen substrate.

Effect of different Amino Acids: Earlier results showed that *P. aphanidermatum* grew best in the media containing casein hydrolysate. Since casein hydrolysate is a mixture of several amino acids and amides, it was decided to determine the growth response in different amino acids. Ten amino acids were used as a source of nitrogen in the basal medium. All amino acids were used at a rate to give an equivalent of 100 µg/ml of nitrogen in the medium. The results are presented in Table 3.

With individual amino acids as the nitrogen source, the growth of *P. aphanidermatum* was poor in most cases except when DL-aspartic acid or L-asparagine were used in the basal medium (Table 3). Least growth was obtained in the presence of glycine, which normally is a good source of nitrogen for most of the fungi.

It was assumed that there might be a difference in the way an amino acid affected the growth of *P. aphanidermatum* in the presence of other amino acids in the basal medium. To test this hypothesis the ten amino acids under study were incorporated in the basal medium to give a total of 100 µg/ml. nitrogen concentration. From these ten amino

Table 3. Mycelial dry weigth of *Pythium aphanidermatum* on media containing different amino acids.

Amino acid *	Dry weight (mg.)	Final pH
DL-aspartic acid	265	5.4
L-asparagine	220	5.4
L-lysine HCl	61	5.8
L-histidine HCl	72	5.6
Glycine	48	5.4
β-alanine	66	5.2
DL-isoleucine	72	5.4
L-threonine	62	5.6
L-tryptophane	76	6.0
DL-methionine	79	5.2

* Each amino acid was used at a concentration equivalent to give 100 μg/ml. of nitrogen.

acids in the basal medium one was omitted at a time, keeping the total nitrogen concentration the same, and the differences in growth were attributed to the missing amino acid. The results are given in Table 4.

Table 4. Mycelial dry weight of *Pythium aphanidermatum* on media containing different combinations of amino acids.

Medium *	Dry weight (mg.)	Final pH
Complete	199	5.6
DL-aspartic acid omitted	129	5.8
L-asparagine omitted	182	6.0
L-lysine omitted	232	5.2
L-histidine omitted	187	5.6
Glycine omitted	179	5.8
β-alanine omitted	191	6.0
DL-isoleucine omitted	203	5.6
L-threonine omitted	281	5.6
L-tryptophane omitted	210	5.4
DL-methionine omitted	172	6.2

* The complete medium consisted of ten amino acids, each in equal proportion to make a total of 100 μg N/ml. in the basal medium.

It may be seen from Table 4 that there was poor growth of *P. aphanidermatum* in the absence of DL-aspartic acid. A considerable increase in the mycelial yield of the organism was observed when the medium was devoid of DL-isoleucine, L-lysine or L-tryptophane, which probably acted as inhibitors. The absence of other amino acids indi-

vidually from a mixture of ten did not influence the growth rate of the organism. The pH of the medium did not change much after the growth and remained in the optimum range.

In another experiment the additive effect of some of the growth inhibiting and growth promoting amino acids was determined by adding these in various combinations. The total nitrogen concentration was kept at 100 μg/ml. in all cases. The results are presented in Table 5.

Table 5. Additive effects of different amino acids on the mycelial dry weight of *Pythium aphanidermatum*.

Nitrogen source	Dry weight (mg.)	Final pH
L-lysine + DL-isoleucine	53	5.2
L-lysine + Glycine	49	5.0
L-lysine + L-histidine	53	5.2
L-lysine + L-tryptophane	46	5.3
DL-isoleucine + Glycine	58	5.1
DL-isoleucine + L-histidine	41	5.3
DL-isoleucine + L-threonine	52	5.2
DL-isoleucine + L-tryptophane	50	5.2
DL-aspartic acid + L-lysine	163	5.5
DL-aspartic acid + DL-isoleucine	177	5.4
DL-aspartic acid + L-asparagine	235	5.6
DL-aspartic acid + L-asparagine + L-lysine	183	5.5
DL-aspartic acid + L-asparagine + DL-isoleucine	191	5.2
L-asparagine + DL-isoleucine + L-lysine	140	5.8
L-threonine + L-tryptophane + Glycine + + L-histidine	81	5.0

A considerable increase in the yield of fungal mycelium was obtained when the medium contained DL-aspartic acid in combination with L-asparagine. Presence of L-lysine or DL-isoleucine in all combinations reduced the growth. When either of these amino acids were added with DL-aspartic acid or L-asparagine, an additive effect was noted. Combination of other amino acids did not enhance much growth.

Discussion.

The response of *Pythium aphanidermatum* to different nitrogen sources varied considerably. Nitrate nitrogen and amino nitrogen sources supported better growth of the organism than the ammonium nitrogen source, while nitrite nitrogen was not assimilated at all. The toxic effect of nitrite nitrogen in acidic ranges is well known (Lilly and Barnett, 1951; Cochrane, 1958). The fact that increased nitrate and

ammonium concentrations in the medium decreased the mycelial yield, while increased concentrations of casein hydrolysate enhanced the growth of the organism, indicated the superiority of the amino sources, and not the ammonium or nitrate sources, for the growth of this organism. Saksena, Jain and Jafri (1952) observed that for most of the *Pythium* species tried by them acetamide, ammonium chloride, sodium nitrate, and urea were poor nitrogen sources, while asparagine and alanine supported good growth.

Among the several amino acids tried for the growth of *P. aphanidermatum,* only aspartic acid and asparagine were best assimilated, while in the presence of other amino acids the growth was poor; least being in glycine. Wolf and Shoup (1943) observed that some species of *Allomyces* were unable to assimalate glycine. Aspartic acid and asparagine have been reported to be good sources of nitrogen for several fungi (Wolf, 1949; Pelletier and Keitt, 1954; Lewis, 1957; Grover and Chona, 1960; Grover, 1964). The fact that absence of aspartic acid in a pool of amino acids did not increase the growth of *P. aphanidermatum* indicated that this organism did not assimilate most of these amino acids properly. It is further strengthened by the fact that several combinations of two or more amino acids did not increase the growth except when aspartic acid or asparagine were added to the medium. Asparagine is a β-amide of aspartic acid and both of these chemicals, particularly aspartic acid, are prime components of transaminating mechanism in many organisms (Kamin and Handler, 1957). It has been observed that α-keto acids of aspartic acid are prominent intermediates in tricarboxylic cycle, and decarboxylation or transamination of aspartic acid results in many amino acids (Davis, 1955). The selective assimilation of aspartic acid and asparagine by *P. aphanidermatum* is indicative of such an endogenous mechanism of decarboxylation and transamination in this fungus.

Summary.

Pythium aphanidermatum assimilated amino and nitrate nitrogen better than ammoniuf nitrogen, while nitrite nitrogen was not utilized. Increased concentration of nitrate or amonium salts decreased the growth, while with the increase in casein hydrolysate concentration increased growth was observed. Among the ten amino acids tried as nitrogen source, only DL-aspartic acid and L-asparagine supported good growth. Poor growth resulted when the fungus was grown on a medium containing all the amino acids except DL-aspartic acid. Combination of various amino acids other than containing DL-aspartic acid and/or L-asparagine did not increase the growth, thus indicating direct assimilation of certain amino acids.

References

Cochrane, V. W., 1958: Physiology of Fungi. J. Wiley, N. York.

Davis, B. D., 1955: Intermediates in amino acid biosynthesis. Advances in Enzymol. **16**, 247—312.

Grover, R. K., 1964: The effect of amino acids on growth and sporulation of *Aspergillus flavus* and their carry-over for subsequent spore germination. New Phytologist **63**, 12—20.

Grover, R. K. and B. L. Chona, 1960: Comparative studies on *Sclerotium rolfsii* Sacc. and *Ozonium texanum* Neal and Wester var. *parasiticum* Thirumalachar. Indian Phytopath. **13**, 118—129.

Horrow, B., 1946: Textbook of Biochemistry. Saunders, Philadelphia.

Kamin, H. and P. Handler, 1957: Amino acid and protein metabolism. Ann. Rev. Biochem. **26**, 419—490.

Kumar, M. and R. K. Grover, 1966: Effect of carbon sources on the growth of *Pythium aphanidermatum* (Edson) Fitz. Indian Phytopath. (in press).

Lewis, R. W., 1957: Amino acid nutrition of **Alternaria solani**. Phytopath. **47**: 121—124.

Lilly, V. G. and H. L. Barnett, 1951: Physiology of Fungi. McGraw Hill, N. Y.

Pelletier, R. L. and G. W. Keitt, 1954: *Venturia inaequalis* (Cke.) Wint. VI. Amino acids as source of nitrogen. Amer. J. Bot. **41**, 362—371.

Saksena, R. K., S. K. Jain and M. H. Jafri, 1952: Sulphur and nitrogen requirements of the genus *Pythium*. J. Indian Bot. Soc. *31*, 281—286.

Wolf, F. T., 1949: Amino acids in the biosynthesis of Penicillin. Mycologia **41**, 403—410.

Wolf, F. T. and C. S. Shoup, 1943: The effects of certain sugars and amino acids upon respiration of *Allomyces*. Mycologia **35**, 192—200.