Nitrogen requirements of three storage rot fungi

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Introduction

Nitrogen is an essential element for fungi. It is not only required for functional and structural purposes but also constitutes a fraction in vitamin and other essential metabolites. Foster (1949) has reported that nitrogen content of mycelium generally lies between 3 and 6 percent which varies with the species, age, nutrition and other factors. In nature, both organic and inorganic forms of nitrogen are available to fungi but as far as utilization is concerned they fundamentally differ from each other in their metabolic potentialities.

During the last two decades many workers including Wolf et al. (1950), Brock (1951), Fergus (1952), Beckman et al. (1953), Converse (1953), Subramanian and Srinivasapai (1953), Tandon and Agarwal (1953), Wolf (1953 and 1955), Tandon and Bilgrami (1954 and 1957), Shirakawa (1955), Leaphart (1956), Lopatecki and Newton (1956), Taber and Vining (1957), Hall (1959), Misra and Mahmood (1960) as well as Saksena and Kumar (1961) have obtained diverse results about the utilization of different nitrogen compounds by the fungi investigated by them.

In the present investigation the effect of various nitrogen compounds on the growth and reproduction of three storage rot fungi has been studied.

Material & Method

Single spore cultures of *Fusarium solani* (Mart.) App. and Wr. (Isolated from bulbs of *Gladiolus*), *Botryodiplodia ananassae* (Sacc.) Pet. (isolated from fruits of *Ananas comosus* Merr.) and *Dothiorella phaseoli* (Maubl.) Petr. et Syd.*) (isolated from tubers of *Solanum tuberosum* L.) were used in the present study. Potassium nitrate of the Asthana and Hawker's medium A (Glucose 5.0 g, KNO_3 3.5 g, KH_2PO_4 1.75 g, $MgSO_4$. $7H_2O$ 0.75 g and distilled water 1 L) was

*) *Dothiorella phaseoli* (Maubl.) Petr. et Syd. in Fedde Rep. nov. spec. Beiheft XLII, p. 241 (1926). — Cr. Petrak in Sydowia XXI. p. 247 (1968).

replaced singly with the different organic and inorganic nitrogen compounds. Pure chemicals (E. Merck or B. D. H.) were used. On the basis of previous investigations, the pH of the medium was adjusted to 5.5, before autoclaving. Twenty five ml of the medium was apportioned in 150 ml Erlenmeyer flasks and were either autoclaved at 15 lb pressure for 15 min in case of inorganic sources or were steam sterilized in case of organic sources.

The media inoculated by the agar disc method and incubated upto 15 days at 25 ± 1° C. Triplicate sets were used in every case and the average dry weights have been recorded.

The degree of sporulation was classified into five categories, viz., excellent, good, fair, poor and absent (nil), on the basis of visual observations. The results were statistically analysed and are recorded in the table.

For grading the dry weights into good, moderate and poor, the general mean (G. M.) of the experiment ± critical difference (C. D.) at 5% level has been reported as moderate (M.) The dry weights higher or lower than the moderate have been designated as good (G) or poor (P) respectively.

Observation & Discussion

The importance of nitrogen in the nutrition of present fungi was exhibited by the fact there was no growth of *Fusarium solani*, *Botryodiplodia ananassae* and *Dothiorella phaseoli* when nitrogen source was excluded from the nutrient solution. This established, that the above fungi were incapable of assimilating atmospheric nitrogen for their growth.

All the three organisms utilized nitrogen from a number of different nitrogen compounds. Significantly good growth of these fungi was recorded on potassium nitrate, peptone, aspartic and glutamic acids, glycine, alanine, valine, leucine, isoleucine, phenylalanine, serine, threonine and proline. Phenylalanine was the best source of nitrogen for *F. solani* and *B. ananassae* while *D. phaseoli* accomplished maximum growth on leucine.

Sodium and potassium nitrites as well as hydroxylamine were toxic to *D. phaseoli* as they did not permit any growth. *B. ananassae* also failed to grow on both the nitrites but it attained very poor growth on hydroxylamine. All of them supported poor growth of *F. solani.*

All the three fungi under investigation differed in the utilization of nitrates. *D. phaseoli* had good growth on all the four of them, *F. solani* grew satisfactorily on potassium, sodium and calcium nitrates while *B. ananassae* had good growth on potassium nitrate only.

The utilization of nitrate nitrogen is believed to be associated with the reduction of nitrate. Natarajan (1958) has suggested a

scheme consisting of a number of steps requiring various enzymes for the utilization of nitrate by *Fusarium vasinfectum*. On the basis of certain enzymic studies as well as from the uncertain occurrence of hydroxylamine in nitrate cultures it has been suggested by Nason (1956) that the inorganic pathway of nitrogen reduction is as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow ? \rightarrow NH_2OH \rightarrow NH_3$$

The unknown intermediate is probably hyponitrite. Wheter this is the sole pathway of nitrate reduction in fungi or not is not yet settled.

All the ammonium compounds were poor sources of nitrogen for *Fusarium solani* and *Botryodiplodia ananassae*, while they were good sources for *Dothiorella phaseoli*. Tandon (1961) and his co-workers have also reported ammonium salts (viz., ammonium chloride, ammonium nitrate and ammonium sulphate) to be unsatisfactory sources for most of the fungi studied by them.

B. ananassae and *D. phaseoli* were incapable of utilizing nitrogen from nitrites of potassium and sodium while *F. solani* accomplished poor growth on these substances.

This behaviour may be explained either on the basis of the presence or absence of the enzyme nitrite reductase or it may be due to the toxic action of nitrite. Cochrane and Conn (1950), Cochrane (1950) and Nord and Mull (1945) have reported nitrite to be toxic in acidic range of pH as it is generally present in the form of undissociated nitrous acid.

Thiourea was very poorly utilized by all the three fungi. Hydroxylamine was toxic to *D. phaseoli* but was poorly utilized by other two organisms. Brock (1951) has reported that both the substances were toxic to the fungi investigated by him. According to Sumner and Sommers (1947) as well as Lardy et al (1949) they are known to be enzyme inhibitors.

Urea was found to be a good source of nitrogen for *D. phaseoli* but it was unsatisfactory for *F. solani* and *B. ananassae*. It has been reported to be a poor source by Uppal et al. (1938), Wooster and Cheldelin (1945) and Tandon (1950) for the fungi investigated by them.

Peptone (a mixture of peptides of varying chain length) is reported to be a good source for a number of fungi studied by Farries and Bell (1930), Ajello (1943), Converse (1953), Marsden (1954), Shirakawa (1955), Misra and Mahmood (1960) as well as Subramanian (1961). Similar results were obtained with the present fungi also.

The two monoamino dicarboxylic acids (viz., aspartic and glutamic acids) were found to be good sources of nitrogen for the organisms under study and in this respect the results were similar to those of

Table showing the average dry weight in mg, sporulation/sclerotial production, final pH of *Fusarium solani*, *Botryodiplodia ananassae* and *Dothiorella phaseoli* on different nitrogen sources.

Nitrogen compounds	*F. solani* Dry wt in mg	Sporulation	Final pH	*B. ananassae* Dry wt in mg	Sporulation	Final pH	*D. phaseoli* Dry wt in mg	Sclerotial development	Final pH
1. Potassium nitrate	71.7 G	Excellent	7.2	63.5 G	Excellent	6.8	61.9 G	Excellent	6.5
2. Sodium nitrate	68.4 M	Excellent	7.1	58.0 P	Good	6.4	61.6 G	Excellent	6.4
3. Calcium nitrate	76.7 G	Good	5.8	51.3 P	Poor	5.4	59.7 G	Fair	5.7
4. Magnesium nitrate	65.1 P	Excellent	7.0	56.5 P	Nil	6.0	69.1 G	Excellent	6.0
5. Potassium nitrite	60.5 P	Excellent	8.2	00.0 —	—	—	00.0 —	—	—
6. Sodium nitrite	59.1 P	Excellent	8.1	00.0 —	—	—	00.0 —	—	—
7. Hydroxylamine	41.3 P	Nil	2.5	16.9 P	Nil	3.7	00.0 —	—	—
8. Ammonium chloride	61.0 P	Good	3.2	43.0 P	Nil	3.3	63.1 G	Nil	2.9
9. Ammonium sulphate	53.4 P	Good	3.1	37.3 P	Nil	3.3	70.5 G	Nil	2.8
10. Ammonium carbonate	60.1 P	Poor	2.5	40.2 P	Nil	3.4	57.5 G	Fair	3.0
11. Ammonium nitrate	53.3 P	Excellent	6.0	30.6 P	Nil	4.0	59.8 G	Fair	3.0
12. Urea	57.9 P	Excellent	7.3	55.7 P	Nil	6.3	61.6 G	Fair	5.7
13. Thio-urea	11.5 P	Nil	5.6	5.6 P	Nil	5.7	12.5 P	Nil	5.6
14. Peptone	90.1 G	Excellent	7.3	77.7 G	Good	7.1	74.3 G	Good	6.6
15. DL-Aspartic Acid	97.1 G	Excellent	9.7	103.3 G	Good	9.0	61.9 G	Poor	5.2
16. L-Glutamic acid	95.0 G	Good	9.6	102.7 G	Good	7.9	69.1 G	Poor	5.3
17. Asparagine	70.2 M	Excellent	8.2	62.0 G	Excellent	7.7	74.0 G	Fair	7.5
18. L-Arginine	62.9 P	Excellent	7.5	66.3 G	Fair	7.3	77.6 G	Excellent	6.4
19. L-Lysine	72.3 G	Good	6.9	67.4 G	Good	3.7	48.3 P	Nil	4.4
20. L-Histidine	84.6 G	Good	6.7	76.5 G	Nil	6.8	40.9 P	Poor	5.2
21. Glycine	71.8 G	Good	7.9	63.5 G	Nil	6.7	68.9 G	Nil	4.6
22. DL-Alanine	75.7 G	Excellent	8.0	77.0 G	Nil	7.0	74.3 G	Nil	6.7
23. DL-Valine	92.1 G	Excellent	7.6	68.0 G	Nil	4.9	66.2 G	Fair	4.5
24. L-Leucine	83.3 G	Excellent	7.6	78.6 G	Nil	6.5	78.1 G	Poor	4.6
25. Isoleucine	84.3 G	Excellent	7.2	80.5 G	Nil	5.5	70.0 G	Excellent	4.5
26. DL-Phenylalanine	109.7 G	Excellent	7.4	121.5 G	Nil	6.9	63.0 G	Poor	4.6
27. DL-Serine	96.6 G	Excellent	8.1	94.7 G	Excellent	7.1	67.9 G	Nil	5.2
28. DL-Threonine	77.3 G	Excellent	7.4	78.9 G	Nil	5.6	69.5 G	Poor	4.7
29. DL-Tryptophane	83.4 G	Excellent	7.5	78.3 G	Excellent	6.9	52.0 P	Good	4.8
30. L-Proline	83.9 G	Excellent	7.8	111.5 G	Good	7.4	77.6 G	Nil	6.4
31. DL-Methionine	45.7 P	Fair	6.4	44.9 P	Nil	5.9	62.0 G	Nil	4.5
32. Creatinine	31.6 P	Fair	5.3	69.8 G	Nil	5.6	44.5 P	Fair	5.4
33. Acetamide	68.7 M	Excellent	6.8	37.9 P	Fair	5.6	43.4 P	Fair	5.6
34. No Nitrogen	00.0 —	—	—	00.0 —	—	—	00.0 —	—	—
General Mean =	68.1			59.4			54.7		

Summary of the dry weight results and conclusions at 5% level of P

F. solani		*B. ananassae*		*D. phaseoli*	
Treatments	Highly significant	Treatments	Highly significant	Treatments	Highly significant
Replicates	Non significant	Replicates	Non significant	Replicates	Non significant
S. E.	0.81	S. E.	0.19	S. E.	0.89
C. D. at 5% level	± 2.3	C. D. at 5% level	± 0.53	C. D. at 5% level	± 2.5

Mosher et al. (1936), Steinberg (1942), Wolf et al. (1950), Brock (1951), Wolf (1955), Tandon and Bilgrami (1957), as well as Strider and Winstead (1960) for a number of fungi investigated by them.

All of the monocarboxylic acids included in the present study (viz., glycine, alanine, valine, leucine, isoleucine, phenylalanine, serine, threonine and proline) were found to be good sources for the growth of all the three organisms. Tryptophane was the only exception because it was good for *F. solani* and *B. ananassae* and poor for *D. phaseoli.*

According to Cochrane (1958) the effect of methionine or cystine was more related to sulphur than to nitrogen metabolism. It appeared to be true for the present organisms also. It was noticed that methionine was poor for the growth of *F. solani* and *B. ananassae* and was good for *D. phaseoli.* The extra amount of sulphur present in methionine may be responsible for such behaviour because the growth of both *F. solani* and *B. ananassae* decreased when the amount of magnesium sulphate was more than 0.75 g per litre (Bhargava & Tandon, 1963). The supply of additional sulphur (from methionine) appears to be mainly responsible for depressing the growth. The adverse effect was not observed on *D. phaseoli* as its growth continued to increase upto 3.0 g of magnesium sulphate per litre and therefore the additional sulphur supplied by methionine did not decrease the growth (Bhargava & Tandon, 1963).

The exact reason for a good or a poor growth on different amino acids depends upon on a number of factors such as permeability, enzymatic capacity of the organism and also to some extent the acidity of the medium.

Gottlieb and Ciferri (1956) while working on *Streptomyces venezuelae* suggested that the difference in the growth on various amino acids may be due different degrees of availability of carbon skeleton of the amino acids for the synthesis of carbohydrates, proteins and fats. Jennison et al. (1955) reported that there was some correlation between the amount of growth of certain Basidiomycetes and the type of structure of amino acids, although this was not consistently true for an individual organism. They suggested that both the environmental factors and intrinsic differences in molecular structure may be involved in the differences noticed in the utilization of various amino acids.

The results of the present study show that the three fungi under study were able to utilize both nitrate and ammonium forms of nitrogen but the inorganic nitrogen sources were generally inferior to some of the amino acids. Wolf (1955) obtained similar results with *Fusarium oxysporum* var. *nicotianae.*

The present fungi could utilize nitrate, ammonium and organic sources of nitrogen and they were unable to fix atmospheric nitrogen. They may, therefore, be placed in group II of the classification proposed by Robbins (1937).

A review of the table shows that *Fusarium solani* sporulated on all substances except on hydroxylamine and thiourea. The sporulation of *B. ananassae* was restricted to a few sources only. The sclerotial production of *D. phaseoli* was observed on a number of compounds. The sporulation or sclerotidal development ranged from excellent to poor and there was no correlation between sporulation and growth because excellent sporulation was found to be associated with good, moderate or poor growth and similarly poor sporulation was also found to be associated with all the three grades of the growth.

Potassium nitrate supported excellent sporulation of all the three fungi. Similar results were recorded by Mix (1933) and Patel et al. (1950) for their organisms. The behaviour on the other nitrates varied with the organism involved.

In general, ammonium compounds suppressed the sporulation or sclerotial development of *B. ananassae* or *D. phaseoli*. Similar results were also obtained by Thind and Randhawa (1957) and Misra and Mahmood (1960) for the organisms studied by them.

The sporulation of *F. solani* was excellent on the nitrites of potassium and sodium, though they supported poor growth only.

Peptone was a favourable source for the sporulation or sclerotial development of the fungi under study. Mathur et al (1950) and Marsden (1954 also found it to be a good source for the sporulation of the fungi investigated by them.

All the three organisms either failed to sporulate or produce any sclerotia on thiourea. Similar results have been reported by Tandon and Grewal (1956) for *Gloeosporium musarum, G. papayae* and *Colletotrichum papayae*.

The sporulation on the different amino acids varied considerably and was dependent on the organism as well as on the amino acid concerned.

In general, the pH changes during incubation were dependent upon the kind of nitrogen source, the extent of growth and the particular organism involved. According to Brock (1951) the pH of any medium containing ammonium salts drops during the incubation period propably due to the differential absorption of the basic group from the medium. During the growth of *F. solani* and *B. ananassae* on glutamic and aspartic acids the pH increased and this may be due to the differential removal of the acidic group. Similar explanation may be given for nitrites of sodium and potassium where the pH increased after the activity of *F. solani*.

Summary

The effect of 33 different nitrogen (both organic and inorganic) sources on growth and reproduction on three storage rot fungi viz., *Fusarium solani* (Mart.) App and Wr., *Botryodiplodia ananassae* (Sacc.) Pet., and *Dothiorella phaseoli* (Maubl.) Petr. et Syd. was studied. In general amino acids supported good growth of the fungi. L-leucine was best for *D. phaseoli* while DL-phenylalanine was best for *F. solani* and *B. ananassae*. Nitrates were good for *D. phaseoli*, satisfactory for *F. solani* while in case of *B. ananassae* only potassium nitrate supported good growth. Nitrites inhibited the growth of *B. ananassae* and *D. phaseoli* while they were poor sources for *F. solani*. All the ammonium compounds were poor sources of nitrogen for *F. solani* and *B. ananassae* while they were good for *D. phaseoli*.

Different sources of nitrogen greatly influenced the reproduction of these fungi. *F. solani* sporulated on all compounds except on hydroxylamine and thiourea. Sporulation of *B. ananassae* was restricted to a few sources only. Sclerotial production of *D. phaseoli* was observed on a number of compounds. In general ammonium compounds suppressed the reproduction of *B. ananassae* and *D. phaseoli*. There was no correlation between growth and sporulation.

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