PHYSIOLOGICAL STUDIES ON SOME MEMBERS OF THE FAMILY SAPROLEGNIACEÆ

III. Nitrogen Requirements*

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Introduction

The essentiality of a proper source of nitrogen, which is an important limiting factor in the nutrition of fungi, has been well recognised. Experimental data on their nitrogen needs have continued to accumulate during the last 15 years. Robbins (1937) has recently pointed out that fungi fall into four groups, when classified on the basis of their ability to assimilate various forms of nitrogen. According to him "Nitrogen fixing organisms" are such as are capable of assimilating nitrogen as gaseous nitrogen, nitrates, ammonium salts and organic nitrogenous compounds. Under the second group come the "Nitrate ammonium organisms" which are capable of assimilating nitrogen as nitrates, ammonium salts and organic nitrogenous compounds but are incapable of assimilating gaseous nitrogen. The third group consists of "Ammonium organisms" which can assimilate nitrogen as ammonium salts and organic nitrogen but are incapable of assimilating gaseous nitrogen and nitrates. The last category comprises the "organic nitrogen organisms" which are capable of assimilating organic nitrogenous compounds only.

The study of various genera belonging to the family Saprolegniaceæ seems to have been much neglected as regards this important factor. Volkonsky (1933, 1934) studied the suitability of some nitrogenous substances on a few of them but he too did not give a relative value of various nitrogenous substances used by him. A little later Leonian and Lilly (1938) found that *Saprolegnia parasitica* along with some others was unable to grow with ammonium nitrate and required an amino acid for its growth.

The present investigation was undertaken with the aim of clarifying and adding to our knowledge the nitrogen needs of some members of the family Saprolegniaceæ, *viz.*, *Achlya* sp., *Brevilegnia gracilis* v. Eek., *Isoachlya anisospora* var. *indica* Sak. et Bhar., *Saprolegnia delica* Coker and *S. monoica* Pringsh. hitherto uninvestigated.

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METHODS

The methods and technique employed in this investigation were the same as described in an earlier paper (Bhargava, 1945). The basal medium consisted of 0·5 gm. each of KH_2PO_4, $MgCl_2 \cdot 6\,H_2O$, 0·17 gm. of Na_2S, 5 gm. of dextrose and 1,000 c.c. of double distilled water. The source of sulphur in the case of *Brevilegnia gracilis* was in the form of K_2SO_4 (0·5 gm. per lit.) instead of Na_2S. Magnesium chloride was prepared by the action of pure hydrochloric acid on magnesium ribbon, the commercial reagent grade chemical (Proanalysis of Merck) being of no value in these experiments, as it contained traces of ammonia.

Because of the prohibitive prices of various amino acids only qualitative experiments were carried out in the first instance. The cultures were grown in culture tubes containing 10 c.c. of the nutrient medium. To compare the relative value of nitrogenous compounds as source of nitrogen only a few representatives were taken for quantitative experiments.

Various nitrogenous compounds (inorganic and organic) were added singly to the basal medium in amounts calculated to furnish 700 mg. of nitrogen per litre. Trihydroxy triethylamine, tyrosin and cystin were tried in 0·1%, 0·1% and 0·05% concentrations respectively because of their low solubility. Peptone was used in 0·1% concentration because of its unknown constitution.

EXPERIMENTAL

To the basal medium were added the following compounds singly before autoclaving :—

Inorganic nitrogen.—

Ammonium nitrate, ammonium chloride, ammonium sulphate, sodium nitrate and sodium nitrite.

Organic nitrogen.—

Amino acids :

(*a*) Mono-amino derivatives of aliphatic mono-carboxylic acids—
Glycin, *d*-alanin, *d*-valin and *l*-leucin.

(*b*) Diamino derivatives of aliphatic mono-carboxylic acids—
d-arginin and *d*-lysin.

(*c*) Mono-amino derivatives of aliphatic dicarboxylic acids—
l-asparatic acid, *d*-glutamic acid and asparagin.

(*d*) Aromatic amino acids—
l-phenyl-alanin and tyrosin.

(*e*) Heterocyclic amino acids—
Histidin, tryptophane and prolin.

(*f*) Thioamino acids—
Cystin and cystein hydrochloride.

Amides : Acetamide.

Amines : Urea and trihydroxytriethylamine.

Proteins : Peptone.

The various media thus obtained were inoculated with the fungi. The basal medium alone served as control. It was found that all the nitrogenous substances, except sodium nitrate, sodium nitrite, glycin, arginin, urea and trihydroxytriethylamine were able to supply nitrogen necessary for the growth of *Achlya* sp., *Isoachlya anisospora* var. *indica* and *Saprolegnia monoica*. In the case of *Brevilegnia gracilis* sodium nitrate, glycin and trihydroxyethyl-amine also supported growth, while acetamide was valueless.

To compare the relative value of some of the easily available nitrogenous compounds, the media were poured in flasks and inoculated with the fungi. Table I gives a résumé of this experiment.

TABLE I

Dry weight (in mg.) of fungal colonies grown on 25 c.c. of the basal medium containing organic and inorganic nitrogenous compounds

Period of incubation = 21 days. Temperature = 25° C.

Compounds	*Achlya* sp.	*B. gracilis*	*I. anisospora* var. *indica*	*S. delica*	*S. monoica*
Ammonium nitrate ..	12·0	20·0	10·0	5·0	25·0
Amm. chloride ..	10·2	10·6	9·5	3·5	21·6
Amm. sulphate ..	9·8	10·0	8·6	4·5	15·0
Sodium nitrate ..	..	18·3	..	..	..
Acetamide ..	5·0	..	4·3	5·0	3·0
Glycin ..	..	27·3	..	..	..
Alanin ..	12·3	32·6	21·6	3·5	6·5
Glutamic acid ..	15·0	23·0	37·3	15·0	29·0
Asparagin ..	5·8	28·3	13·3	15·0	10·0
Basal medium (control) ..	..	..	..	..	..

The results summarised in Table I show that of all the nitrogenous substances tested, glutamic acid is generally the best and acetamide the poorest source of nitrogen.

Effect of sodium acetate on the utilisation of glycin.—Enhancement of growth of *Leptomitus lacteus*, a watermold, by the addition of glycin in a medium suggested to Schade (1940) that it might prove an available nitrogen source if any suitable carbon could be supplied. He found that an acetate, which is an oxidisable substrate, provides a very favourable source for glycin utilisation in the case of *L. lacteus*. To see if acetate would induce growth of the organisms in this case too (where a good source was already present), the following media were prepared :

1. KH_2PO_4 .. .. 0·5 gm.
   $MgCl_2 \cdot 6H_2O$ .. .. .. 0·5 „
   Na_2S .. .. 0·15 „
   Glycin .. .. 3·0 „
   Distilled water .. .. .. 1,000 c.c.
2. Medium 1+glucose .. .. 5·0 gm.
3. Medium 2+sodium acetate .. 2·0 „
4. Medium 1+sodium acetate .. 2·0 „

These were inoculated with *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia monoica* and *S. delica*. On examining the cultures after seven days of inoculation, it was found that there was no growth.

Effect of molybdenum on the utilisation of nitrates.—Steinberg (1937) in his extensive and well-balanced studies stressed the importance of trace elements in the nutrition of fungi. He found that molybdenum was required in a greater degree by the organisms when a nitrate and not ammonia or organic nitrogen was the source. The following media were prepared to see the possible effect, if any, of molybdenum on the utilisation of nitrate :

1.	KH_2PO_4	0·5 gm.
	$MgCl_2 \cdot 6\,H_2O$	0·5 ,,
	Na_2S	0·15 ,,
	Dextrose	5·0 ,,
	$NaNO_3$	4·2 ,,
	Distilled water	1,000 c.c.

Achlya sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* which showed no growth on a medium containing $NaNO_3$ were grown on media 1 and 2. It was found that there was no growth in any case.

Discussion

Since the fungi used in the present study are unable to grow on a synthetic medium lacking in the source of nitrogen, it is evident that nitrogen is essential for the growth of these organisms. As to the form of nitrogen suitable for their growth, *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* are unable to utilise nitrite or nitrate, but show good response to ammonium as well as organic nitrogen. Therefore, they very well fit in the "Ammonium organisms" placed separately by Robbins (1937). Steinberg (1937) has shown that molybdenum is essential for the activation of nitrate reductase in the reduction processes whereby nitrates are reduced to ammonia. That the non-utilisation of nitrates is not due to the absence of molybdenum is shown clearly by the absence of growth of these organisms on the medium to which molybdenum had been added in the form of sodium molybdate. *Brevilegnia gracilis* behaves differently and is able to utilise nitrates in addition to ammonium and organic nitrogen. It comes under "Nitrate ammonium organisms". This difference may easily be explained on the basis of the different habitat of the fungi ; *B. gracilis* is a parasite on the roots while the other forms are watermolds.

The general opinion about the availability of nitrite nitrogen is that it is toxic for the growth even in dilute solutions, and that if the fungi use it, they do so with difficulty (Ohtsuki, 1936 ; Sakaguchi and Wang, 1936 ; Wang, 1937 and others). Of the 25 fungi used by them, Leonian and Lilly (1938) obtained fair amount of growth of only one organism, *viz.*, *Blakeslee trispora* with sodium nitrate as source of nitrogen.

Of the organic nitrogen, proteins which are the result of a combination of different amino acids are necessary for the building up of the body of an organism. This holds true for fungi as well. Since the organisms studied above are able to utilise nitrogen from NH_4NO_3 or a single amino acid as the only source of nitrogen in a nutrient medium, it can safely be concluded that they manufacture their own amino acids from these substances. Leonian and Lilly (1938) have reported that *Blakeslee trispora, Phycomyces nitens, Pythium oligandrum* and *P. polymastum*, etc., require only one favourable amino acid as the source of nitrogen for good growth. These organisms resemble *Pythium arrhenomanes, P. deliense, P. graminicolum* and *P. mamillatum* (Saksena, 1940) which are able to grow with NH_4NO_3 or one amino acid.

Amines are generally poor source of nitrogen. Schade (1940) also found them valueless for *Leptomitus lacteus.* Volkonsky (1933, 1934) reported that acetamide was not assimilated by *Saprolegnia dioica* while glycocoll and urea were utilised by this fungus.

The best growth on glutamic acid can be attributed, as already explained by Waksman and Lomanitz (1925), to the much larger ratio of the carbon to the nitrogen and that glutamic acid is very favourable to respiration, resulting in the formation of very little volatile acids. This is further explained by the fact that when the ratio between carbon and nitrogen is high, the amount of ammonia produced will be less because the fungus will continue to grow and derive its energy from the available carbohydrate, and ammonia which is a waste product in carbon metabolism will be utilised resulting in greater amount of growth.

That the failure of *Achlya* sp., *Isoachlya anisospora* var. *indica Saprolegnia delica* and *S. monoica* to grow on glycin is not due to the absence of proper carbohydrate or any oxidisable substance is clear from the experiment where these fungi failed to grow on a medium to which acetate had been added in the presence of glycin.

SUMMARY

Achlya sp., *Isoachlya anisospora* var. *indica, Saprolegnia delica* and *S. monoica* are unable to utilise nitrite or nitrates as source of nitrogen but thrive on ammonium and organic nitrogenous compounds. Glutamic acid serves as the best source, while acetamide is the poorest. *Brevilegnia gracilis* is able to obtain nitrogen from nitrates as well. Addition of molybdenum does not help in the assimilation of nitrates. Glycin, which is a good source of nitrogen for *B. gracilis*, is valueless for the growth of others, and remains so even in the presence of an oxidisable substrate.

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LITERATURE CITED

Bhargava, K. S. (1945) .. "Physiological studies on some members of the family Saprolegniaceæ. II. Sulphur and Phosphorus requirements", *Proc. Ind. Acad. Sci.*, 1945 (in press).

Leonian, L. H., and Lilly, V. G. (1938) "Studies on the nutrition of fungi. I. Thiamin, its constituents, and the source of nitrogen," *Phytopath.*, **28,** 531–38.

Ohtsuki, T. (1936) .. "Untersuchungen über die Nitritverwendung und die Nitrat reduktion bei Schimmelpilzen," *Japan Jour. Bot. (Tokyo)*, **8,** 269–93.

Robbins, W. J. (1937) .. "Assimilation by plants of various forms of nitrogen," *Amer. Jour. Bot.*, **24,** 243–50.

Sakaguchi, K., and Wang, Y. (1936) "Studies on the assimilation of nitrites by fungi," *Bull. Agr. Chem. Soc. Japan*, **12,** 63–69.

Saksena, R. K. (1940) .. "The nutrition of some species of the genus *Pythium* on synthetic liquid media," *Proc. Nat. Acad. Sci., India*, **10,** 1–13.

Schade, A. L. (1940) .. "The nutrition of *Leptomitus lacteus*," *Amer. Jour. Bot.*, **27,** 376–84.

Steinberg, R. (1937) .. "Rôle of molybdenum in the utilisation of ammonia and nitrate nitrogen by *Aspergillus niger*," *Jour. Agr. Res.*, **55,** 891–902.

Volkonsky, M. (1933) .. "Sur les conditions de culture et le pouvoir de synthese de *Saprolegnia* sp. Etudé qualitative de l'alimentation carbonéé, azotéé et sulphurèa," *Ann. Inst. Pasteur*, **50,** 703–30.

——— (1934) .. "Sur les nutrition de quelques champignons saprophytes et parasites," *ibid.*, **52,** 76–101.

Waksman, S. A., and Lomanitz, S. (1925) "Contribution to the chemistry of decomposition of proteins and aminoacids by various groups of micro-organisms," *Jour. Agr. Res.*, **30,** 263–81.

Wang, Y. (1937) .. "Assimilation of nitrites by fungi. III," *Jour. Agr. Chem. Soc. Japan*, **12,** 877–84 (Original not seen).