PHYSIOLOGICAL STUDIES ON SOME MEMBERS OF THE FAMILY SAPROLEGNIACEÆ

Part II. Sulphur and Phosphorus Requirements*

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INTRODUCTION

The importance of the knowledge of the nutritive relations of a fungus for cultural purposes is well known. The foremost consideration which forms a part of the general programme for its study is the formulation of a synthetic nutrient medium of known definite composition suitable for its growth.

A review of the available literature shows that adequate attention has not been paid to the study of sulphur requirements of the fungi. The author is of opinion that sulphur plays a very important rôle in their nutrition. An unsuitable sulphur compound in the nutrient medium may lead to erroneous results. Leonian and Lilly (1938), for example, while studying the effect of various nitrogenous substances on the growth of Achlya conspicua, Aphanomyces camptostylus, Isoachlya monilifera, Saprolegnia mixta and S. parasitica among others, used a nutrient medium containing KH₂PO₄, MgSO₄, NH₄NO₃, dextrose and distilled water. The fungi named above failed to respond to the above medium even in the presence of thiamin, but showed growth in the presence of an aminoacid, viz., l-cystin. From this they concluded that this aminoacid and not NH₄NO₃ was the proper source of nitrogen. The work of several investigators (Volkonsky, 1933, 1934; Dayal, 1942; and Bose, 1943) has amply proved that Saprolegniaeaceous fungi are unable to utilise sulphates but readily take up cystin and sulphides. It appears to the author that in the above case the nutrient medium lacked in a proper source of sulphur which was accidentally supplied by l-cystin, when added as a substitute for ammonium nitrate.

Fungi have been cultured on a large number of media containing various sulphur compounds. Recently Steinberg (1941) from his extensive study of sulphur nutrition of Aspergillus niger was able to conclude that sulphur was reduced to sulphonylate prior to its conversion to organic sulphur.

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and that this organism was unable to utilise sulphides and disulphides. Other biological data obtained by various workers show that fungi have been successfully grown on various sulphur compounds (Armstrong, 1921; Kossowicz and Loew, 1912; Volkonsky, 1933, 1934) including sulphides and disulphides. Fischer (Lwoff, 1932) has classified the organisms into two categories: (1) "Euthiotrophe" which can obtain their sulphur from "SO₄" ions and (2) "Parathiotrophe" which cannot utilise "SO₄" ions. The present investigation was, therefore, undertaken to study the relation between constitution and assimilation of sulphur compounds in the case of Achlya sp., Brevilegaria gracilis v."Eek., Isoachlya anisospora (DeBary) Coker, var. indica Sak. et Bhar., Saprolegnia delica Coker and Saprolegnia monoica Pringsh.

Along with the investigation of sulphur requirements, experiments dealing with the utilisation of phosphorus compounds were also performed.

Methods

The fungi were grown either in culture tubes (1.5 x 15 cm.) or in 150 ml. Erlenmeyer flasks containing 10 and 25 c.c. of a nutrient medium respectively. The basal medium, which will hitherto be referred to as medium A, in the case of experiments dealing with sulphur requirements consisted of 0.5 gm. each of KH₂PO₄, MgCl₂.6 H₂O, 2 gm. of NH₄NO₃, 5 gm. of dextrose and 1000 c.c. of double-distilled water. Various sulphur compounds were added singly to the basal medium so as to furnish 25 mg. of sulphur per litre. In the case of hydrogen sulphide, the gas was passed for 5 minutes through 200 c.c. of the basal medium. The composition of the basal medium in the case of experiments dealing with phosphorus requirements was as follows:—MgCl₂.6 H₂O—0.5 gm., NH₄NO₃—2.0 gm., dextrose—5 gm., Na₂S—0.15 gm. and double distilled water—1000 c.c. Sulphur supplied in the case of Brevilegaria gracilis was in the form of K₂SO₄ (0.5 gm. per lit.) since it supports better growth of the organism. The amount of various phosphorous compounds added to the basal medium was such as to furnish 115 mg. of phosphrous per lit.

The solutions were sterilised in an autoclave at 15 pounds pressure for 15 minutes. The hydrogen-ion concentration of the nutrient media was adjusted with NaOH and HCl so that it was approximately 7 after autoclaving. Three or more replicates were used in each of the several experiments, all of which were carried on at 25°C. The incubation period varied from 10–21 days. Only guaranteed reagents (either of Mercks or British Drug House) were used.

The stock cultures were maintained on the basal medium solidified with Difco bacto-agar. Material for inoculum was taken from the margin
of a young colony taking care that the size of the inoculum was equal (4 mm.) in all cases. For transfers from a tube to a flask containing the same medium, some mycelium was dissected out inside the tube with the help of a platinum needle and transferred aseptically. A particular substance was thought suitable only when the fungus grew on it by subsequent transfers.

Only pure cultures were employed throughout all the experiments reported below. Whenever a culture showed bacterial contamination, it was discarded.

Dry weights were determined by washing the mycelium thoroughly with distilled water on a weighed filter-paper and drying it in an electric oven at 60° C. for 72 hours. The dried mats were removed from the oven to the desiccator, cooled and weighed rapidly on an analytical balance to constant weight. Since there was not much difference in the weights of colonies produced in replicate cultures, only the average values have been tabulated.

Only Pyrex glassware, thoroughly cleaned with chromic-sulphuric acid mixture and washed twice with distilled water, was used throughout.

**Experimental**

**Sulphur Requirements**

Medium A with and without the addition of various sulphur compounds given in Table I was inoculated with the fungi. The results are tabulated in Table I.

**Table I**

*Dry weight (in mg.) of the fungal colonies grown on medium A with and without sulphur compounds*  
*(Time of incubation = 21 days)*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Achlya sp</th>
<th>B. gracilis</th>
<th>I. anisopora var. indica</th>
<th>S. delic</th>
<th>S. menoica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium sulphate</td>
<td>..</td>
<td>..</td>
<td>22.3</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Sodium bisulphide</td>
<td>..</td>
<td>..</td>
<td>20.0</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>..</td>
<td>..</td>
<td>16.3</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Sodium hyposulphite</td>
<td>4.0</td>
<td>23.3</td>
<td>10.6</td>
<td>8.2</td>
<td>15.3</td>
</tr>
<tr>
<td>Sodium sulphide</td>
<td>10.0</td>
<td>20.3</td>
<td>13.7</td>
<td>10.0</td>
<td>17.3</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>4.0</td>
<td>20.0</td>
<td>6.8</td>
<td>9.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Potassium persulphate</td>
<td>..</td>
<td>..</td>
<td>17.3</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>2.0</td>
<td>20.3</td>
<td>8.0</td>
<td>9.8</td>
<td>15.7</td>
</tr>
<tr>
<td>Sodium dithionate</td>
<td>..</td>
<td>2.8</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Cystin</td>
<td>11.0</td>
<td>15.0</td>
<td>11.0</td>
<td>12.3</td>
<td>28.7</td>
</tr>
<tr>
<td>Cysteine hydrochloride</td>
<td>4.3</td>
<td>8.3</td>
<td>6.0</td>
<td>9.7</td>
<td>15.5</td>
</tr>
<tr>
<td>Thiourea</td>
<td>5.7</td>
<td>6.6</td>
<td>7.6</td>
<td>1.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Medium A (control)</td>
<td>..</td>
<td>2.6</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
</tbody>
</table>
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It is seen from the results summarised in Table I that Achlya sp., Isoachlya anisospora var. indica, Saprolegnia delica and S. monoica did not grow on medium A and were unable to utilise sulphur in the form of potassium sulphate, sodium bisulphite, sodium sulphite, potassium persulphate and sodium dithionate. Of the inorganic compounds tried, sodium sulphide supported the maximum growth, while cystin served the best source among the organic compounds. Brevilegnia gracilis showed good growth in all cases except on medium A and the medium containing sodium dithionate: traces of growth were present on these two. The dry weights of mycelium varied according to the sulphur compounds in the following diminishing order—hydrogen sulphide, sodium hyposulphite, potassium sulphate, sodium thiosulphate, sodium sulphide, sodium bisulphite, potassium persulphate and sodium sulphite.

**Phosphorus Requirements**

The basal medium with and without the addition of various phosphorus compounds given in Table II were inoculated with the fungi. The results are summarised in Table II.

**Table II**

*Dry weight (in mg.) of the fungal colonies grown on basal medium with and without phosphorous compounds*  
(Time of incubation = 21 days)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Achlya sp</th>
<th>B. gracilis</th>
<th>L. anisospora var. indica</th>
<th>S. delica</th>
<th>S. monoica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium dihydrogen</td>
<td>5.0</td>
<td>36.7</td>
<td>16.0</td>
<td>10.0</td>
<td>13.3</td>
</tr>
<tr>
<td>phosphate</td>
<td>55.0</td>
<td>68.3</td>
<td>53.3</td>
<td>51.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Casein</td>
<td>11.0</td>
<td>42.0</td>
<td>20.0</td>
<td>17.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td></td>
<td>traces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal medium (control)</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
</tbody>
</table>

It is evident from the data obtained in Table II that the fungi did not grow on the basal medium lacking in phosphorous compounds, but were able to utilise phosphorus both from inorganic and organic sources.

**DISCUSSION**

That sulphur is essential for the growth of the organisms is shown by the absence of growth on medium A. The slight growth of Brevilegnia gracilis obtained on medium A, and on medium A containing sodium dithionate is due to the traces of sulphur (0.002%) present as impurities in the form of “sulphates” (SO₄) in both MgCl₂·6 H₂O and NH₄NO₃ (Proanalysi of Merck). It seems that even this small amount is sufficient for the growth
of _B. gracilis_ which is able to assimilate sulphur in the form of sulphates. Results obtained for _Achlya_ sp., _Isoachlya anisospora_ var. _indica_, _Saprolegnia delica_ and _S. monoica_ are in general agreement with those obtained by Volkonsky (1934) for some members of the family Saprolegniaceae. He found that they were unable to use sulphates but utilised thiosulphates, sulphhydryl and sulphides. The results of Leonian and Lilly (1939) indicate that organic sulphur is necessary for the growth of _Saprolegnia mixta_, _S. parasitica_, _Achlya conspicua_, _Isoachlya monilifera_ and _Aphanomyces camptostylus_ (Steinberg, 1939, p. 335). Schade (1940) found that _Leptomitus lacteus_ and _Apodachlya brachynema_, sewage water-molds, were able to reduce sulphates to satisfy their sulphur requirements. Persulphate, which served as a good source of sulphur in the case of _Brevilegnia gracilis_, proved a favourable source of sulphur for _Aspergillus niger_ (Armstrong, 1921; Steinberg, 1941) and _Penicillium glaucum_ (Armstrong, 1921) also. Dithionate, which is a valueless compound as a source of sulphur in the present case, behaves similarly with _Aspergillus niger_. These fungi, except _Brevilegnia gracilis_, are thus able to utilise the inorganic sulphur in a reduced form. Oxidised forms like sulphates and sulphites are not assimilated.

Phosphorus is equally important for the growth of the organisms is clear from the results summarised in Table II. The fungi do not grow on a basal medium lacking in phosphorous compounds. No comparative value can be attached to these data since casein and nucleic acid may contain some growth stimulatory factors in addition to an extra amount of available carbon and nitrogen.

**Summary**

_Achlya_ sp., _Brevilegnia gracilis_, _Isoachlya anisospora_ var. _indica_, _Saprolegnia delica_ and _S. monoica_ are unable to grow on a nutrient medium in the absence of sulphur. These fungi except _Brevilegnia gracilis_ cannot obtain sulphur from sulphate, sulphite, bisulphite, persulphate and dithionate but thrive well with hydrogen sulphide, sodium sulphide, hyposulphite, thiosulphate, cystin, cysteine hydrochloride and thiourea. _Brevilegnia gracilis_ can utilise sulphur from all the substances tried except dithionate. All the five fungi are able to utilise both inorganic and organic phosphorous compounds, in the absence of which they show no growth.

**Acknowledgments**

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