**Effect of phosphate on lipid metabolism in Fusarium oxysporum**

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The effect of different phosphate levels in the growth medium on lipid metabolism in *Fusarium oxysporum*, was studied. An increase in the phosphate level led to an increase in the phosphorus content of the mycelium, as well as in that of total lipids. However, higher phosphate concentrations, had no profound effect on the total lipid percentage. Among phospholipid compounds phosphatidic acid (PA) and phosphatidylinositol were the most susceptible while phosphatidyl choline (PC) was found to be the least affected by changes in the phosphate content of the medium. In the pulse and chase experiments, higher specific activities were observed for PA, PGP and cardiolipin (CL) fractions.

**INTRODUCTION**

*Fusarium oxysporum* Schlecht. has already been reported to be an excellent fat synthesizer, having as high as 28.7 percent of total lipids (Bhatia et al. 1973). Some aspects of lipid metabolism of this mould under various culture conditions, and the use of some inhibitors have already been discussed (Bhatia, Arneja 1978a, b). The present study deals with certain metabolic aspects of various lipids as affected by the phosphate level in the growth medium, both under the deficiency and the excess of phosphate.

**MATERIALS AND METHODS**

*Fusarium oxysporum* was grown in the liquid synthetic medium, described by Chaahl and Gray (1969) as a still culture, at 25 ± 1°C (Bhatia et al. 1973). Five different phosphate levels were used: 0.25, 2.50, 12.50 and 25.0 g KH₂PO₄ per litre of the growth medium.
For the radioactivity incorporation the fungus was grown in the growth medium containing the 10 μCi of 1-C¹⁴-acetate (sodium salt) per litre for the pulse period (8 days). Then the mats were washed 3-4 times with distilled water to eliminate superficial contamination of the radioactivity. Next they were macerated, after which the fresh medium was added and the fungus was allowed to grow for various periods of chase. At the end of the required incubation period, the mycelium was harvested, washed with distilled water and macerated in a washing blender. The lipids were extracted by the method of B I g h y and D y e r (1959), and fractionated into individual lipid classes by the monodimensional double development t.l.c. technique (B h a t i a, A r n e j a 1978b). The bands of different lipid fractions were visualized by iodine vapour, scrapped off and eluted (R a h e j a et al. 1973). The radioactivity was measured by liquid scintillation spectrometer (K a t e s 1972). Phospholipid phosphorus was estimated by the A m e s’s (1966) method. Phospho- and non phospholipids were fractionated by preparative t.l.c. using solvent system of G a r d n e r (1968) acetone: acetic acid: water (80:20:1 v/v) followed by fractionation of phospholipids by using solvent system of – chloroform: methanol: 7N ammonia solution (65:25:5 v/v).

RESULTS AND DISCUSSION

The studies showed that an increase in the phosphate in the growth medium led to an increase in the phosphorus content of the mycelium as well as of total lipids (Table 1). Total mycelium production per flask was however, adversely affected e.g. the value of 562.2 mg mycelial dry matter at 0.25 g K H₂PO₄ per litre decreased to 153.2 mg/flask as the K H₂PO₄ level of the medium was raised to 25 g per litre. Total lipid percentage in the mycelium, however, increased from 26.6% at 0.25 g K H₂PO₄ per litre to 33.04% at 12.50 g K H₂PO₄ per litre. Further rise in the phosphate level of the medium had an adverse effect on the lipid percentage too. Phosphorus content of both the mycelium and of total lipids was found to increase significantly with the growth of phosphate level of the medium. Similar regularities have also been observed by R a h e j a et al. (1983) for Pythium irregulare. The detailed analysis of phospholipid fraction obtained by preparative t.l.c. using solvent system of – acetone: acetic acid: water (100:2:1 v/v) followed by rechromatography using solvent system of – chloroform: methanol: 7N ammonia solution, revealed six phospholipid classes viz PA, PI, PC, PE, PGP, and CL. Among them Pa and CL showed a rapid increase in their relative percent value with the growth of phosphate level of the medium while PI followed the reverse trend. PC and PE, manifested only an insignificant increase with an increase in the phosphate level from 0.25 to 2.50 g K M₂PO₄ per litre. Further raising of the phosphate level has similar effects on PC, but the rate
Table 1
Effect of different concentrations of KH₂PO₄ in the medium on the quantity and composition of lipids

<table>
<thead>
<tr>
<th>KH₂PO₄ (g/L)</th>
<th>Total dry mycelium / flasks/mg</th>
<th>Total lipids / flasks/mg</th>
<th>Total mycelial phosphorus / mg/g</th>
<th>Lipid phosphorus / mg/g</th>
<th>Phospholipids / %</th>
<th>Percent phospholipid class</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>562.2</td>
<td>159.9</td>
<td>26.60</td>
<td>5.5</td>
<td>13.1</td>
<td>32.77</td>
</tr>
<tr>
<td>2.50</td>
<td>560.0</td>
<td>160.8</td>
<td>28.70</td>
<td>13.5</td>
<td>33.85</td>
<td>2.48</td>
</tr>
<tr>
<td>12.50</td>
<td>336.1</td>
<td>114.3</td>
<td>33.04</td>
<td>12.0</td>
<td>39.55</td>
<td>8.71</td>
</tr>
<tr>
<td>25.00</td>
<td>153.2</td>
<td>47.3</td>
<td>30.89</td>
<td>15.0</td>
<td>39.67</td>
<td>9.18</td>
</tr>
</tbody>
</table>

PA - Phosphatidic acid  PE - Phosphatidyl ethanolamine
PI - Phosphatidyl inositol  PGP - Polyglycerophosphatide
PC - Phosphatidyl ethanolamine  CL - Cardiolipin

of PE synthesis was adversely affected. It is postulated that CL may represent a labile pool of PA. Under conditions where the synthesis of PA is too rapid for its conversion into other phosphatides such as PI, PE and PC, the surplus PA is channelled into this pool. The decline in PGP concentration may indicate its utilization for the synthesis of other phosphatides via PA as shown below:

Precursors of PA

PE → PA ← PGP

PC ↓ PI

Table 2
Rate of decline of radioactivity from prelabelled /with sodium acetate-1-⁴¹C/ mycelium in total lipids and individual lipid fractions after different intervals of chase

<table>
<thead>
<tr>
<th>Incubation Period (days)</th>
<th>Culture conditions</th>
<th>Total lipids</th>
<th>Specific activity (cpm/mg lipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FA</td>
<td>PI</td>
</tr>
<tr>
<td>8+0²</td>
<td></td>
<td>461</td>
<td>782</td>
</tr>
<tr>
<td>8+4</td>
<td></td>
<td>280</td>
<td>284</td>
</tr>
<tr>
<td>8+8</td>
<td>Normal growth</td>
<td>181</td>
<td>288</td>
</tr>
<tr>
<td>8+12</td>
<td></td>
<td>143</td>
<td>149</td>
</tr>
<tr>
<td>8+16</td>
<td></td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>8+4</td>
<td></td>
<td>348</td>
<td>900</td>
</tr>
<tr>
<td>8+4</td>
<td>Phosphorus starvation</td>
<td>319</td>
<td>197</td>
</tr>
<tr>
<td>8+8</td>
<td></td>
<td>314</td>
<td>138</td>
</tr>
<tr>
<td>8+12</td>
<td></td>
<td>305</td>
<td>114</td>
</tr>
<tr>
<td>8+16</td>
<td></td>
<td>284</td>
<td>116</td>
</tr>
</tbody>
</table>

² - Figures before and after * indicate pulse and chase periods, respectively.
The normal growth medium contained 2.5 g KH₂PO₄ per litre both in pulse and chase periods.
The phosphorus starvation medium contained 0.25 g KH₂PO₄ during pulse and nil KH₂PO₄ during chase period.

PA - free fatty acids; SGP - steryl glycosides and pigments; MDS - monoglycerides, diglycerides and free steryls; TG+SGH - triglycerides-steryl esters and hydrocarbons.
The results of pulse and chase experiment on the lipid metabolism under normal and starvation conditions (Table 2) indicated that the rate of decline in specific activity of lipids was relatively rapid under normal conditions as compared with starvation conditions. In the latter case the observed decrease in specific activity of lipids might result from certain inter conversions between non-lipid and lipid and lipid components. Significant decrease in specific activity values of lipids was observed only during the initial periods of starvation and the subsequent rate of decline was of a much lower magnitude. A more rapid rate of radioactivity dilution of a particular fraction would indicate a more rapid of metabolism of that class. Therefore, low labelling pattern may reflect either extensive turnover or minimal incorporation of the precursor into a particular fraction. The specific activity of individual lipid fractions indicated that, in general, both under normal and starvation conditions the metabolism of various fractions was very active during zero time to 4 days chase, which may point to either the repair process of cell injured during maceration or the synthesis of new cells when the mycelium is transferred to a fresh medium. Similar observations have also been made by Richard et al. (1972) who has pointed out that during the recovery of Salmonella typhimurium from thermal injury, while phosphatidyl ethanol anime and phosphatidyl glycerol were synthesized in concentrations comparable to those at normal growth, the amount of cardiolipin synthesized during the recovery period was greater than that obtained from normal cells.

Since, under both normal growth and starvation conditions, the other environmental conditions (temperature, pH and medium composition) were not altered, it is not surprising that in such a controlled environment structural stability of lipids was not affected during starvation. Reports on this aspect of lipid metabolism in the literature are somewhat variable. For example, Wheatham (1922) and Ingaram (1939) while working on Mycobacterium phlei and Bacillus cereus, respectively, have observed the utilization of lipid reserves of the body. On the other hand, Bions and Davies (1963), while working on Sarcina lutea and Pseudomonas aeruginosa, have failed to find many evidence for such use of the reserve fat, Kostiw et al. (1972), while working on Arthrobacter crystallopoeties have shown that structural stability of lipids is not destroyed when the bacteria are cultured under the stress of starvation.

Furthermore, to find out the effect of the induced stress of a very low phosphorus content in the medium on the phospholipid composition, and to determine whether these effects were of reversible or irreversible nature, an experiment was planned, in which the fungus after being grown in normal and a low phosphorus medium was transferred back to the medium containing the normal concentration of phosphate or to medium containing various levels above and below the usual phosphate concentration for the growth of this fungus.
The data (Table 3) showed the specific activity distribution pattern for the individual lipid classes. There was a restoration trend for the phospholipid classes when the phosphate level of the medium was also restored. The specific activity values of the individual phospholipid classes decreased relatively more rapidly than the non-phospholipid classes by replenishing the phosphate supply of the growth medium. This indicated that the conditions again favoured the new growth, and the rate of phospholipid synthesis was again accelerated. It is concluded that in the phosphate-deficient medium, the machinery for the biosynthesis of the phospholipids is temporarily shut off, since no sustained phospholipid biosynthesis is possible under such conditions. Ehen the phosphorus supply is restored, the mechanism for phospholipid biosynthesis is again switched on and the pattern of phospholipid in the organism is restored near to that prevailing at the normal phosphate supply to the organism.

**REFERENCES**


