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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 on 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 phosphatidyl inositol were the most susceptible while phisphatidyl 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 (B h a t i a 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 (B h a t i a, A r n e j a 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 C h a h a l and G r a y (1969) as a stil culture, at $25 \pm 1°C$ (B h a t i a et al. 1973). Five different phosphate levels were used: 0.25, 2.50, 12.50 and 25.0 g KH_2PO_4 per litre of the growth medium.

For the radioactivity incorporation the fungus was grown in the growth medium containing the 10 μCi of 1-C^{14}-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 ratioactivity. 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 l i g h and D y e r (1959), and fractioned 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 fractioned 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 KH_2PO_4 per litre decreased to 153.2 mg/flask as the KH_2PO_4 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 KH_2PO_4 per litre to 33.04% at 12.50 g KH_2PO_4 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 KM_2PO_4 per litre. Further raising of the phosphate level has similar effects on PC, but the rate

Table 1

Effect of different concentrations of KH_2PO_4 in the medium on the quantity and composition of lipids

KH_2PO_4 /g/L/	Total dry mycelium/ flask/mg/	Total lipids/ flask/mg/	Total lipid /%/	Mycelial phospho-rus /mg/g/	Lipid phospho-rus /mg/g/	Phospho-lipids /%/	Percent phospholipid class					
							PA	PI	PC	PE	PGP	CL
0.25	562.2	159.9	26.60	5.5	13.1	32.77	2.48	11.71	50.53	26.14	6.31	2.83
2.50	560.0	160.8	28.70	10.0	13.5	33.85	5.74	6.67	51.48	27.13	6.09	2.89
12.50	336.1	114.3	33.04	12.0	15.0	39.55	8.71	6.95	52.63	23.53	5.59	3.59
25.00	153.2	47.3	30.89	15.0	15.9	39.67	9.18	7.07	52.54	19.49	5.36	6.36

PA - Phosphatidie acid
PI - Phosphatidyl inositol
PC - Phosphatidyl choline
PE - Phosphatidyl ethanolamine
PGP - Polyglycerophosphatide
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 channalled into this pool. The decline in PGP concentration may indicate its utilization for the synthesis of other phosphatides via PA as shown below:

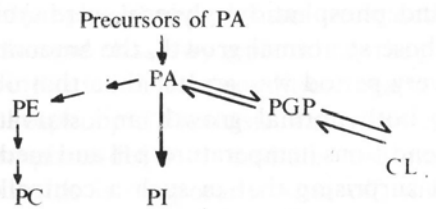

Table 2

Rate of decline of radioactivity from prelabelled /with sodium acetate-1-C^{14}/ mycelium in total lipids and individual lipid fractions after different intervals of chase

Incubation Period /days/	Culture conditions	Total lipids	Specific activity /cpm/mg lipids/								
			PA	PI	PC	PE	PGP	CL+FFA	SGP	MDS	TG+SEH
8+0[a]	Normal growth	461	782	394	385	368	313	664	579	669	140
8+4		280	284	191	193	205	242	421	335	275	110
8+8		181	288	136	105	152	193	275	250	185	98
8+12		143	149	118	94	109	172	185	248	169	80
8+16		103	104	107	84	161	125	175	213	114	69
8+0	Phosphorus starvation	348	900	163	339	338	117	436	760	297	105
8+4		319	197	128	319	143	158	211	245	308	100
8+8		314	138	124	339	218	205	186	244	300	92
8+12		309	114	120	342	196	198	168	226	106	77
8+16		284	116	122	296	145	136	169	204	74	57

a - Figures before and after + indicate pulse and chase periods, respectively.
The normal growth medium contained 2.5 g KH_2PO_4 per litre both in pulse and chase periods
The phosphorus starvation medium contained 0.25 g KH_2PO_4 during pulse and nil KH_2PO_4 during chase period
FFA - free fatty acids; SGP - steryl glycosides and pigments; MDS - monoglycerides, diglycerides and free steryls; TG+SEH - triglycerides+steryl esters and hydrocarbons

The results of pulse and chase esperiment 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 cither the repais process of cell injured during maceration or the synthesis of new cells when the mycelium is transfered to a fresh medium. Similar observations have also been made by R i c h a r d 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, W h e t h a m (1922) and I n g r a m (1939) while working on *Mycobacterium phlei* and *Bacillus cereus,* respectively, have observed the utilization of lipid reserves of the body. On the other had, R i b b o n s and D a w e s (1963), while working on *Sarcina lutea* and *Pseudomonas aeruginosa,* have failed to find many evidence for such use of the reserve fat, K o s t i w 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.

Futhermore, to find out the effect of the induced strees 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.

Table 3
Radioactivity distribution pattern in total lipids and individual lipid classes after different intervals of chase at different concentrations of KH_2PO_4

KH_2PO_4 /g/L/	Incubation period /days/	Specific activity /cpm/mg lipids/									
		total lipids	PA	PI	PC	PE	PGP	CL+FFA	SGP	MDS	TG+SEH
2.5	8	461	782	394	385	368	313	664	579	669	140
2.5 -2.5	8+8	181	288	136	105	152	193	275	250	185	98
2.5 -2.5 -2.5	8+8+8	103	104	107	84	161	125	175	213	114	69
2.5 -0.0	8+8	123	425	283	382	298	210	444	470	515	90
2.5 -0.0 -0.0	8+8+8	172	410	271	378	291	208	434	450	510	80
0.25	8	348	900	163	339	338	117	436	760	297	105
0.25-0.0	8+8	314	138	124	339	218	205	186	244	300	92
0.25 -0.0 -0.0	8+8+8	284	156	122	296	145	136	169	204	74	57
0.25 -0 -0.50	8+8+8	76	115	111	198	120	116	104	101	60	40
0.25 -0 -2.5	8+8+8	140	90	81	180	118	104	90	90	50	30
0.25 -0 -12.50	8+8+8	91	85	80	178	115	98	70	85	48	30

The data (Table 3) showed the specificactivity 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 phosphlipids 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.

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