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Phosphate mediated changes in fatty acid composition in Neurospora crassa.

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Abstract

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From our earlier studies on the effect of inorganic phosphate on alterations in phospholipids (Nair and Chhatpar, 1983, Neurospora Newsletter 30: 11) and changes in sugar uptake in Neurospora crassa (Savant, Parikh and Chhatpar, 1982, Experientia 38: 310-311), we suggested that phosphate might play an important role at the membrane level with respect to uptake and permeability functions. Further, we were interested in seeing the effect of inorganic phosphate on membrane fatty acid composition since alterations in fatty acid composition have been shown to result in changes in ion permeability and enzyme activity (Davis and Silbert, 1974, Biochim. Biophys. Acta 373: 224; Dekruyff, et al., 1973, Biochim. Biophys. Acta 298: 479).

The synthetic liquid medium employed for the growth of N. crassa (wild type, carotenogenic) contained per litre: glucose, 50 g; trisodium citrate, 2.5 g; $(NH_4)_2SO$ 2.5 g; $MgS0_4.7H_2O$, 0.5 g; $ZnS0_4.7H_2O$, 2.5 mg; $FeCl_3$, 5.0 mg; $CaCl_2$, 10 mg; and biotin 100 pg. The pH was adjusted to 5.6. 'High phosphate' condition indicates the addition of KH_2PO 1.0 g% whereas 'Low phosphate' condition indicates the addition of 0.01 g% KH_2PO_4 to the above medium. These phosphate conditions did not change the pH of the medium. Growth temperature was 30° C. Culture density at harvest was determined by drying mycelial mats at 50° C to constant weight: for the low phosphate culture dry mat weight was 0.24 g/50 ml flask, and for the high phosphate culture it was 0.28 g/50 ml flask.

TABLE I

Effect of inorganic phosphate on percentage fatty acid composition in Neurospora crassa

Type of atty acid	High phosphate (KH_2PO_4:1g%)	Low phosphate (KH_2PO_4:0.01g%)
C_{14}	18.87	64.16
C_{16}	16.02	9.17
$C_{16:1}$	6.66	ND
C_{18}	6.27	ND
$C_{18:1}$	6.27	5.24
$C_{18:2}$	27.34	17.07
$C_{18:3}$	18.58	4.36

ND : Not detectable.,

The extraction of lipids was carried out using chloroform:methanol (2:1) (Folch, Less and Stanley, 1957, J. Biol. Chem., 226: 497). Gas-liquid chromatographic analysis of the fatty acids was carried out after preparation of methyl esters and comparing the retention times of reference standards.

Significant changes were observed in the culture grown under high and low phosphate conditions of growth. The percentage of C_{14} fatty acids was found to be considerably lower in high phosphate as compared to low phosphate grown cultures (Table I). However, C_{16}, $C_{16:1}$, C_{18}, $C_{18:2}$ and $C_{18:3}$ were in greater percentage in high phosphate grown cells, The percentage of $C_{18:1}$ fatty acids however, did not register much change.

The alterations in the fatty acid composition along with changes in phospholipids under low and high phosphate conditions further suggests the influence of phosphate at the membrane and hence at the permeability level.

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