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Phosphate mediated regulation of carbohydrate metabolism in *Neurospora crassa*.

Our earlier studies had indicated an increase in the activity of malate dehydrogenase under low phosphate conditions of growth (S. Savant, N. Parikh and H.S. Chhatpar, 1982 Experientia 38: 310-311). This has been substantiated by our present studies where two forms of cytosolic malate dehydrogenase were observed under low phosphate conditions.

In the present study, attempts were made to see the changes in the molecular forms of malate dehydrogenase and protein profiles as revealed by polyacrylamide gel electrophoresis as well as changes in enzyme activities when the culture was subjected to phosphate starvation by growing in a normal medium and transferring to a phosphate deficient medium.

N. crassa (wild type, carotenogenic) was grown under high and low phosphate conditions as described earlier (Nair and Chhatpar, 1983, Neurospora Newsl. 30:ll). For phosphate starvation, mats grown for 48 h in synthetic medium were washed and transferred to medium completely devoid of phosphate for a further 24 h. Polyacrylamide gel electrophoresis was carried out by the method of Davis (B.J. Davis, 1964, Ann. NY Acad. Sci. 112:404). Activity staining for malate dehydrogenase was carried out as described earlier (Nautiyal, Chhatpar and Modi, 1980, Ind. J. Expt. Biol. 18: 362). Methods for preparation of cell-free extracts and assay of malate dehydrogenase, isocitrate lyase and FDP aldolase were the same as described earlier (Savant et al., 1982, Experientia 38: 310-311).

Two forms of cytosolic malate dehydrogenase were observed under low phosphate conditions while high phosphate conditions resulted in the disappearance of one form of enzyme (Fig. 1). Thus high phosphate conditions may be regulating the enzyme at the level of synthesis or stability. Further a variety of other soluble proteins also registered a marked difference as observed by polyacrylamide gel electrophoresis (Fig. 2).

The significant influence of high phosphate conditions led us to study the effect of phosphate deficiency in the medium on the activities some enzymes of carbohydrate metabolism. Malate dehydrogenase was triggered to a higher level under phosphate starvation conditions. Similar results were obtained with isocitrate lyase (Table I). Earlier, a number of phosphate metabolizing enzymes in N. crassa were reported to be highly derepressed conditions of phosphate limitation. These include an alkaline phosphatase (Burton and Metzenberg,

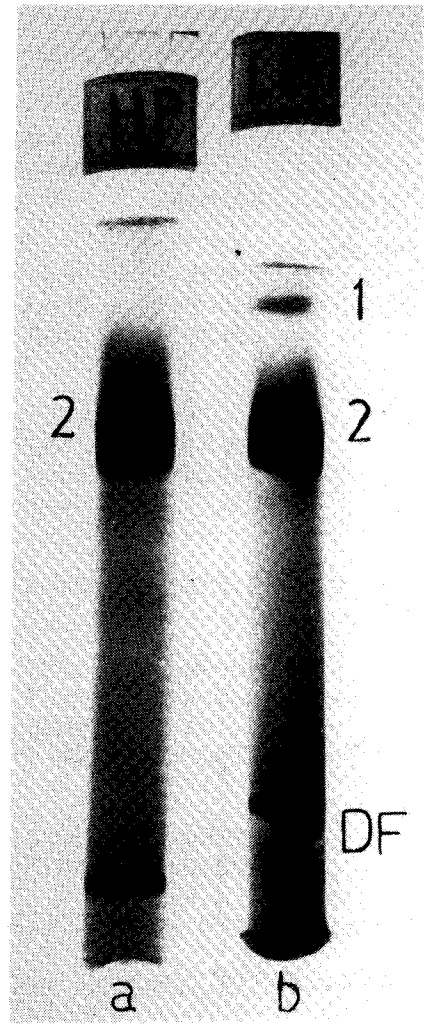

Fig.1 -- Activity staining of polyacrylamide gels for malate dehydrogenase from extracts of N. crassa grown under: a) High phosphate condition b) Low phosphate condition. DF indicates Dye Front

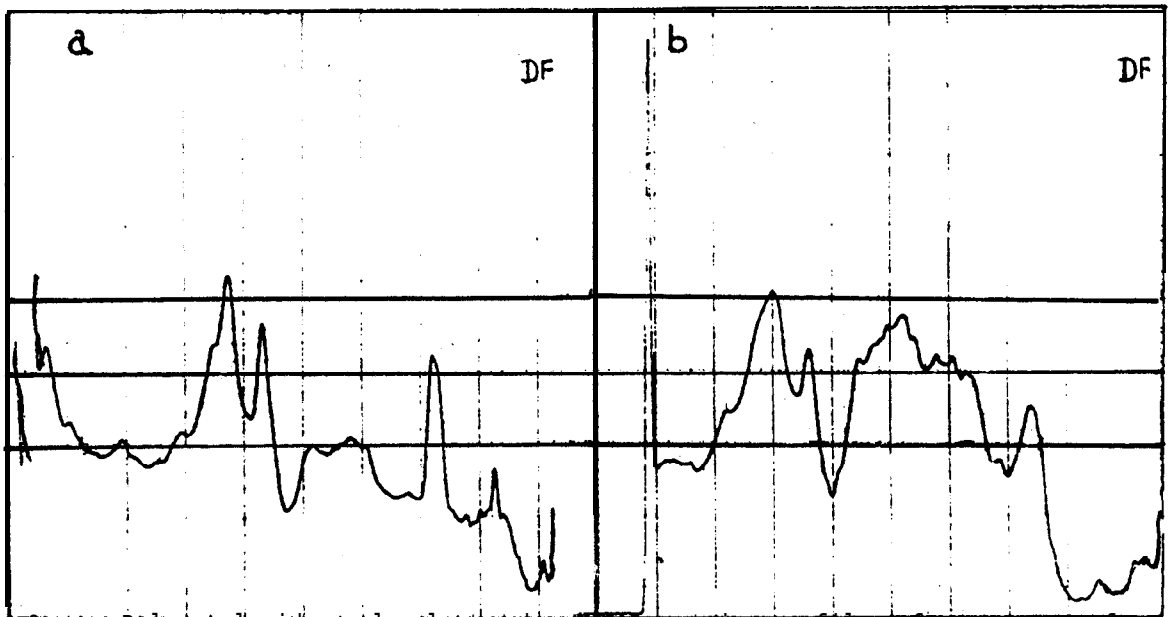

Fig. 2 -- Polyacrylamide gel electrophoresis protein profile of extracts of N. crassa grown under: a) Low phosphate condition b) High phosphate condition.

TABLE: I

Effect of phosphate starvation on enzyme activities in Neurospora crassa

Enzyme	Control (Units/mg protein)	Phosphate Starvation (Units/mg protein)
Malate dehyrogenase	19.9	112.4
Isocitrate lyase	104.4	537.4
FDP adolase	119.3	9.8

1974, J. Biol. Chem, 249: 4679-4688), an acid phosphatase (Nelson, Lehman and Metzenberg, 1976, Genetics 84: 183-192), a phosphate permease (Lowendorf and Slayman, 1975, Biochem. Biophys. Acta 413 95-103) and nucleases (K. Hasunuma, 1973, Biochim. Biophys. Acta 319: 288-293) . The activity of FDP aldolase, however, showed a marked decrease in activity under conditions of phosphate starvation (Table I).

These studies indicate that a specific metabolic set-up is induced in starvation conditions and the regulation of metabolism is considerably altered by the availability of inorganic phosphate in the growth medium. - - - Department of Microbiology, Faculty of Science, M.S. University of Baroda, Baroda 390 002, India.