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Reissig and Kinney reported the role of Ca^{+2} in the induction of apical branching in N. crassa STL74A (Reissig and Kinney 1983 J. Bact. 154:1397-1402). Slayman et al. (1976 Biochim. Biophys. Acta 426:732-744) had suggested that spontaneous localized depolarization events could lead to localized Ca^{+2} entry and hence branching. In the present study, attempts were made to determine the influence of calcium (Ca^{+2}) on carbohydrate metabolism and carotenogenesis in N. crassa.

N. crassa (wild type, carotenogenic) obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India was grown as described earlier (Nair and Chhatpar 1983 Neurospora Newsletter 30:11). Ca^{+2} was added to the synthetic medium devoid of Ca^{+2} as $CaCl_2$ (anhydrous) at the desired concentration. Calcium deficient, calcium optimal and calcium supraoptimal conditions indicate no addition, addition of 10 ug/ml and 100 ug/ml or above to the growth medium, respectively. Methods for the preparation of cell-free extract, assay of FDP aldolase, isocitrate lyase, G6P dehydrogenase and protein were the same as described earlier (Savant et al. 1982 Experientia 38:310-311). Amylase was assayed according to the method of Bernfeld (Bernfeld, P. Meth. Enzymol. 1:149). Thin layer chromatography was carried out on 0.25 mm silica gel G plates (Ranboxy Co.). The solvent system used was 20% ethyl acetate in methylene chloride. Carotenoids were estimated according to the method of Davies (B.H. Davies, In:Chemistry and Biochemistry of Plant Pigments (T.W. Goodwin, Ed.) Academic Press. pg. 389)

N. crassa grown under calcium deficient condition showed lower activity of extracellular amylase as compared to calcium optimal and supraoptimal conditions (Table 1). Calcium deficient cultures however showed higher activities of FDP aldolase and isocitrate lyase as compared to calcium optimal and supraoptimal conditions. No significant change was observed in the activity of FDP aldolase from calcium optimal to supraoptimal conditions. However, in the case of isocitrate lyase, the activity was found to be decreased in supraoptimal as compared to optimal conditions.

Table 1: Effect of Ca^+2 on the activities of extracellular amylase, FDP aldolase, isocitrate lyase and G6P dehydrogenase in N. crassa.

Growth conditions	Extracellular amylase (U/50 ml)	FDP aldolase	Isocitrate lyase	G6P dehydrogenase
			(U/mg protein)	
Calcium deficient	150	193	799	397
Calcium optimal (10 ug/ml)	280	120	440	322
Calcium supraoptimal (100 ug/ml)	261	126	381	348
Calcium supraoptimal (1000 ug/ml)	339	129	327	359

The mechanism of the effect of calcium on enzymes is not known. Whether calcium influences the activity of enzyme(s) or changes the rate of synthesis or changes the level of cyclic AMP which subsequently alters the level or activity of enzyme(s) is not known. Calcium mediated activation of amylase has been reported by Takegi et al. (1971 In:The Enzymes (P.D. Boyer, Ed.) Academic Press 5:235). In other studies in a number of instances, antagonistic regulatory roles of Ca^+2 and cyclic AMP have been suggested (M.J. Berridge 1975 Adv. Cyclic Nucleotide Res. 6:1-98; Rasmussen and Goodman 1977 Physiol. Rev. 57:421-509). Ealier, Flavell and Woodward had demonstrated that isocitrate lyase is subjected to catabolite repression (Flavell and Woodward 1971 J. Bacteriol. 105:200). In the present studies, if calcium changes the level of cyclic AMP at all, then the possibility exists it can affect the synthesis of isocitrate lyase.

Calcium did not affect the activity of G6P dehydrogenase when added in the growth medium. G6P dehydrogenase is known to provide reducing power for the biosynthesis of lipids. The level of G6P dehydrogenase was not found to be affected whereas the level of carotenoids was significantly reduced under supraoptimal as compared to calcium deficient and calcium optimal conditions (Table 2.). This suggests that the effect of calcium may be on some of the enzymes(s) of a carotenogenic pathway. In order to ascertain if there was accumulation of any intermediate(s) in calcium supraoptimal conditions, thin layer chromatography of extracts of carotene from the Ca^+2 supraoptimal and calcium deficient cultures was carried out and the plates were then checked for fluorescence under UV light. The extract of calcium supraoptimal grown culture showed a distinct band which was not so prominent in the calcium deficient culture.

Table 2: Effect of Ca^+2 on carotene production in N. crassa.

Growth conditions	Carotenoids (ug/g mat wet weight)
Calcium deficient	25
Calcium optimal (10 ug/ml)	34
Calcium supraoptimal (100 ug/ml	6

Data obtained in this study indicate the influence of Ca^+2 in the growth medium on some enzymes of carbohydrate metabolism and the production of carotenoids in N. crassa. These studies on the regulatory effects of calcium on biochemical changes could be useful in boosting primary metabolism which can then trigger secondary metabolism. The desired products of secondary metabolism can potentially be increased by maintaining a suitable concentration of Ca^+2 in the growth medium. - - - Dept. of Microbiology, Faculty of Science, M.S. University of Baroda, Baroda 390002, India.