ADDENDUM

SUMMARY OF MAJOR FINDINGS

Metabolism of carbon sources present in nodules and its regulation were studied in free-living cells of root-nodule bacteria of Cicer arietinum. Carbon nutrition of free-living root-nodule bacteria revealed that Rhizobium utilized a broad range of carbon sources whereas Bradyrhizobium was fastidious in nature.

Assays of key enzymes of carbohydrate metabolic pathways in glucose grown BICC 620 revealed a differential availability of the EMP, ED and PP pathways at various phases of growth. Enzyme profiles of hexose or succinate grown cells of BICC 620 revealed that the enzymes of the EMP, ED and PP pathways were inducible and succinate mediates repression of hexose catabolizing enzymes.

Growth of BICC 620 in glucose - succinate mixture exhibited a diauxic pattern and succinate was found to be the preferred carbon source. cAMP was found not to be involved in the regulation of glycolytic enzymes.

Outer membrane protein (OMP) profiles of succinate or glucose-grown cells of BICC 620 showed differences in banding patterns and presence of unique bands during growth on each of these carbon sources. The majority of bacteroid OMP bands were in common to those of succinate grown cells.

Cells of BICC 620 became transformed and pleomorphic in shape in medium containing succinate as carbon source. During diauxic growth

on glucose-succinate, bacteroid like morphology of cells of BICC 620 was apparent during the first log phase. A gradual conversion of cells to rod structure was observed with the onset of second log phase of diauxie.

During growth on acetate repression/inactivation of glycolytic enzymes was observed. Isocitrate lyase, a key enzyme of glyoxylate pathway was induced in root-nodule bacteria during growth on acetate. Low level of the enzyme was also present in glycerol grown cells of Rhizobium. Malate synthase was produced constitutively even during growth on hexoses or succinate. However, hexoses or succinate were favoured over acetate as carbon source. Addition of succinate caused a catabolite type of repression of isocitrate lyase activity synthesized by BICC 620 during growth on acetate. Addition of cAMP to a concentration of 5 mM in these cells relieved catabolite repression mediated by succinate and restored 75% of isocitrate lyase activity found during growth on acetate alone.

Glucose starvation caused decrease in catabolic activities and increase in anabolic activities of carbohydrate metabolic enzymes, glucose starvation also caused induction of isocitrate lyase activity to provide increased level of C-4 compounds for increased gluconeogenesis. Glucose supplementation to these cells restored the enzymes to their original levels.

One to four carbon alcohols also served as the carbon sources for root-nodule bacteria. Presence of alcohols caused induction of alcohol

dehydrogenase and isocitrate lyase activities. At a concentration exceeding optimum level, alcohols exerted toxic effect as revealed by increased generation time of cells of Rhizobium and Bradyrhizobium.

Future research prospect :

Highest availability of the EMP pathway during stationary phase of growth of Rhizobium may possibly be due to low oxygen tension. This contension may be verified by growing the strain in an atmosphere of low oxygen tension and measurement of the enzyme activities.

Further research is needed to ascertain membrane permeability during growth on succinate when OMP profile of Rhizobium is similar to that of bacteroids. It may be interesting to check if any specific OMP protein is responsible for maintaining the rigidity of bacterial structure.

During succinate-mediated catabolite repression of ICL activity it will be interesting to see if there is any transient repression followed by permanent repression of the enzyme.

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