SUMMARY

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Studies on the metabolism of carbohydrates and of certain other carbon sources known to be present in nodules and the regulation of carbohydrate metabolic pathways were carried out in free-living cells of root nodule bacteria of Cicer arietinum with a hope to gain knowledge as to the operation of these pathways in bacteroids.

Initially, carbon nutrition of free living Rhizobium and Bradyrhizobium of Cicer arietinum was studied and compared with that of the defined strains of root nodule bacteria. Nutritional characteristics of fast- and slow-growing strains of Cicer were similar to those of the other defined strains of fast- and slow-growing root-nodule bacteria respectively. Fast-growing strains utilized a broad range of carbon sources including hexoses, pentoses, disaccharides, trisaccharides, organic acids of the TCA cycle and also acetate efficiently. However, an aromatic compound, benzoate was not utilized by most of the strains of Rhizobium. On the other hand, strains of Bradryrhizobium of Cicer arietinum as well as other slow-growing strains were found to be fastidious in their carbon nutrition. These were unable to utilize disaccharides, rhamnose, raffinose and the TCA cycle intermediates such as

citrate and oxalacetate.

Growth kinetic studies of a representative strain, Rhizobium sp. (Cicer arietinum) BICC 620, using optimum concentrations of mannitol (1%), glucose (1%), gluconate (0.5%) and succinate (0.8%) revealed that the generation times of the strain were 3.5, 4.2, 4.6 and 3.6 hours respectively in these carbon sources and the strain reached its stationary phase at about 25 hours in all the carbon sources. In gluconate containing medium the lag phase was extended to about 10 hours while it was only 5 hours in the other carbon sources.

Assays of key enzymes of central carbohydrate metabolic pathways in the cell-free extracts prepared from cells of different phases of growth of BICC 620 in glucose medium indicated a differential availability of the EMP, ED and PP pathways during various phases of growth. Activities of the enzymes of both the ED and PP pathways were much higher in the cells in early log phase as compared to those in the stationary phase of growth. In contrast, the activities of the enzymes of the EMP pathway were higher during the stationary phase of cell growth than in the other growth phases.

Enzyme profiles of BICC 620 grown on hexoses or succinate as carbon sources support that succinate mediates repression of hexose catabolizing enzymes. Enzymes of the EMP, ED and PP pathways were found to be present in glucose,

gluconate and mannitol grown cells but not in succinate grown cells. The enzymes were inducible in the presence of hexoses. Further studies involving a number of strains of Rhizobium and Bradyrhizobium revealed that succinate mediated repression of glycolytic enzymes and their induction in presence of glucose was also true in strains of Bradyrhizobium as well as in the other strains of Rhizobium. In the strains of Bradyrhizobium the PP pathway was not operative even in the presence of hexoses as was revealed by the absence of the enzyme 6-PGD. The TCA cycle enzymes were constitutively synthesized irrespective of the carbon but a marginal increase in activities of these enzymes in succinate grown cells was noted.

Under anaerobic condition the generation time of the strain BICC 620 is about 1.8 days. Low levels of the enzymes of the EMP, ED and PP pathways and the TCA cycle as well as of gluconeogenesis were detected in the cells under anaerobicity indicating simultaneous operation of catabolic as well as anabolic pathways in these cells.

On the other hand, extracts of bacteroid revealed substantial levels of enzymes of glycolytic pathways as well as of gluconeogenesis. A high level of the TCA cycle enzymes were detected in the bacteroid extracts suggesting the operation of the TCA cycle as the primary metabolic route for energy supply in bacteroids.

BICC 620 grown in medium containing a mixture 0.2% succinate plus 0.5% glucose as carbon source exhibited a diauxic pattern of growth. Succinate was used in the first phase and glucose in the second phase of diauxie as revealed from the kinetics of glucose consumption. Enzymes of glycolytic pathways were found to be practically absent during first phase of diauxie but reappeared during the second phase providing evidence of succinate repression of glucose transport and catabolite repression. cAMP was found not to be involved in the regulation of expression of glycolytic enzymes as its presence failed to increase the levels of enzymes of the EMP, ED and PP pathways relieving succinate mediated repression.

Outer membrane proteins (OMPs) of succinate and glucose grown cells resolved in SDS-polyacrylamide gels displayed that about 50% of the proteins including a thick band of 45 KD protein were in common between the cells. However, two protein bands of 47 and 25 KD were unique to glucose grown cells while several bands of protein ranging from 15 KD to 75 KD in M.W. were unique to succinate grown cells. OMP profiles of cells from first log phase and second log phase of diauxic growth on succinate-glucose mixture as carbon source were quite similar to those of succinate grown cells and glucose grown cells respectively. The OMP profile of bacteroids had majority of the protein bands in common with

those of succinate grown cells while a few others were in common with those of glucose grown cells.

Cells of BICC 620 became transformed and bacteroid like with pleomorphic shapes in medium containing succinate as carbon source. The cells were familiar rod shaped during growth on glucose. During diauxic growth on succinate plus glucose mixture bacteroid like morphology of cells of BICC 620 was apparent during first log phase of diauxie when succinate served as the carbon source. A gradual conversion of cells from bacteroid like morphology to rod structure was observed with time with the onset of second log phase of diauxic growth.

Only low levels of enzymes of the EMP, ED and PP pathways provided evidence for poor operation of glycolytic pathways in presence of acetate as carbon source and indicated acetate mediated repression/inhibition of glycolytic enzymes. Gluconeogenesis was favoured during acetate metabolism.

Isocitrate lyase (ICL), a key enzyme of glyoxylate pathway was induced both in Rhizobium and Bradyrhizobium during growth on acetate but not on mannitol, glucose, gluconate, succinate or oxalacetate. Low level of ICL activity in the strains of Rhizobium but not in the strains of Bradyrhizobium was also evident during growth on glycerol. Malate synthase (MS), the other key enzyme of glyoxylate pathway was found to be synthesized constitutively in cells grown on hexoses or

succinate with a marginal increase in the cells grown on acetate. It was found that activities of enzymes of glyoxylate pathway were higher in strains of Rhizobium sp. (Cicer arietinum) and in R. leguminosarum biovar viceae than in other strains of Rhizobium or Bradyrhizobium.

No activity of ICL was apparent in cells in their mid log phase of growth on hexoses or succinate. During late stationary phase the activity of the enzyme appeared possibly when carbon source in the cultures became exhausted. There was a differential availability of glyoxylate pathway at different phases of growth of Rhizobium on acetate and ICL activity was found to be highest in cells at their late log to early stationary phases of growth.

ICL activity of the cells was lost over a period of time when acetate grown cells were transferred to a medium containing glucose. In medium with mixed carbon sources, hexoses or succinate were found to be preferred over acetate as carbon source. This resulted in diauxic growth curve and ICL activity was induced during second phase of diauxic growth presumably due to the use of acetate as carbon source.

During the growth of the strain BICC 620 on acetate when succinate was added ICL activity decreased and reached a basal level within the next ten hours. Addition of 5 mM cAMP to the culture at the time of addition of succinate helped

to maintain at least 75% of ICL activity found in acetate grown cells. It was believed that succinate exerted a catabolite type of repression on the activity of ICL which was antagonized by cAMP. Upon addition of succinate during the growth of BICC 620 on acetate, growth rate also changed and the growth curve became steeper. Presence of 5 mM cAMP resisted the change in the growth rate of the cells.

During non-growing condition activities of glucose catabolic enzymes decreased and of anabolic enzymes increased even in the presence of glucose in Rhizobium sp. (Cicer arietinum) BICC 620. Carbon starvation accentuated these effects on the enzymes. The activities of hexose inducible enzymes of the EMP, ED and PP pathways decreased to a significantly low level upon carbon starvation for 24 hours, in contrast to those of the TCA cycle enzymes which are constitutive. Glucose starvation probably caused increased gluconeogenesis and the induced operation of glyoxylate pathway as was evident from the appearance of ICL activity. MS activity increased to provide increased level of C-4 substrate for gluconeogenesis. Glucose supplementation to the starved cells reversed the effects of starvation and restored the activities of the enzymes almost to their original levels found before starvation.

Ethanol and propanol were best utilized by the strains of Rhizobium and Bradyrhizobium. Methanol and butanol were low in order of preference for utilization of alcohol as carbon source. Presence of alcohols as sole carbon source caused induction of ADH and ICL enzymes. Gluconeogenesis was also favoured in alcohol grown cells. Activities of catabolic enzymes of glycolytic pathways and of the TCA cycle were detected but at a lower level in the alcohol grown cells as compared to the hexose grown cells. At a concentration exceeding optimum level alcohols exerted toxic effects which was reflected in increased generation time of cells of both Rhizobium and Bradyrhizobium.