J. Phytol. Res. **22**(2) : 283-286, 2009

METABOLISM OF HEXANE BY *RHIZOBIUM* SP. (*CICER ARIETINUM*) BICC620

NARAYAN C. MANDAL[1]* and PRAN K. CHAKRABARTTY
Department of Microbiology, Bose Institute, P 1/12 CIT Scheme VII-M, Kolkata-700 054, West Bengal, India.
[1] Department of Botany, Visva Bharati, Santiniketan-731 235, West Bengal, India.
*mandalnc@rediffmail.com

Activities of key enzymes of the Embden Meyerhof Parnas-, Entner Doudoroff-, and pentose phosphate-pathways as well as key enzymes for gluconeogenesis and tri carboxylic acid cycle were assayed from cell free extracts (CFE) of hexane grown culture of *Rhizobium* sp. (*Cicer arietinum*) BICC 620. Level of activities of the enzymes assayed indicate either a very poor or no operation of glycolytic pathways from the CFE, whereas, operation of TCA cycle was moderate as significant level of activities of key enzymes of the pathway is detected from hexane-grown cells of the strain. Cells also operate gluconeogenesis comparatively at a higher rate as indicated by the substantial specific activity fructose-1,6-bisphosphatase and malic enzyme from the CFE.

Keywords: Gluconeogenesis; Glycolysis; Hexane; *Rhizobium;* TCA-cycle.

Introduction

Of the total biologically fixed nitrogen root-nodule bacteria and in particular *Rhizobium* spp. and its related members contribute the highest for agricultural improvement[1]. The *Rhizobium* species isolated from chick pea (*Cicer arietinum*) is also very important because this is third most widely grown legume crop in India as well as in the world. Taxonomically this is also thought to warrant the formation of new separate species[2]. As the reaction of biological nitrogen fixation is ATP and reductant requiring endergonic one, under different situations rhizobia behave differently to operate the central energy metabolic pathways to provide the necessary metabolic energy[3-5]. Although rhizobia grow and fix N_2 under microaerophilic situation within root-nodules as bacteroids, but most of the time they have pass their life as soil bacteria because leguminous plants including chickpea are short lived covering only 3-4 months a year. So, for a major time of a year rhizobia do not get readily available carbon sources like sugars or dicarbxylates from root-nodule and depend only onto soil ingredients which include aromatics, alcohols and even hexanes[6]. This time they become very much energy conscious, use their storage materials and maintain a balance between catabolism and anabolism. Under such situation of carbon starvation, induction of gluconeogenic enzymes is reported in the strain[7]. Induction of other anapleurotic pathways like glyoxylate pathway for gluconeogenesis is also reported in *Saccharomyces cerevisiae*[8], and *Acinetobacter calcoaceticus*[9]. So, in the present investigation an attempt has been taken to find out the availability of metabolic pathways which are normally operated by hexane grown cultures of a fast growing *Rhizobium* sp. (*Cicer arietinum*) BICC 620 in a chemically defined medium.

Material and Methods

A fast growing strain of *Rhizobium* sp. BICC 620 originally isolated from root nodules of *Cicer arietinum* was obtained from our Culture Collection, Kolkata. The strain was maintained by routine transfer on yeast extract mannital agar[10]slopes. The cultures were grown from a 2% inoculum on a rotary shaker (120 rpm) at 28°C in 100 ml nephalometric flasks containing 40 ml of Sherwood's SY medium[11] containing 0.6% n-hexane as sole carbon source and 1% glucose. To get this optimum concentration a range of different concentrations of hexane were tried for the growth of the strain (Fig.1). Growth of the strain was followed by measuring the turbidity in a Klett-Summerson photoelectric colorimeter with a red filter. The cells were harvested at their mid-log phase *i.e.,* 6-days of growth (Fig. 2) by centrifugation at 10,000xg for 10 min at 4°C. The pellet was washed thrice by 50 mM phosphate buffer at pH 7.0. The method of disruption of cells was done using ultrasonic needle probe in a Braun Sonicator model 1510 to obtain cell-free extract (CFE) which has been previously described[12]. All enzyme assays were carried out at room temperature (26 ± 2°C). Published

Table 1. Specific activities (n moles of substrate consumed/min^{-1}/mg^{-1} protein) of the key enzymes of carbohydrate metabolism in cell-free extracts of *Rhizobium* sp. (*Cicer arietinum*) BICC 620 grown on hexane (0.6%) or glucose (%).

Enzymes	Hexane-grown cells	Glucose grown Cells
Phosphofructokinase	0.8	22.1
Fructose bis-phosphate aldolase	1.1	23.8
Glyceraldehyde-3-phosphate-dehydrogenase	9.2	27.4
"ED-enzymes" *	1.2	30.9
6-phosphogluconate dehydrogenase (NADP)	0.0	64.9
Fructose-1,6-bisphosphatase	31.9	37.1
Malic enzyme	41.1	42.3
Malate dehydrogenase	75.0	115.0
Isocitrate dehydrogenase	64.0	147.3
Isocitrate lyase	5.9	2.0

* : Combined activity of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase.

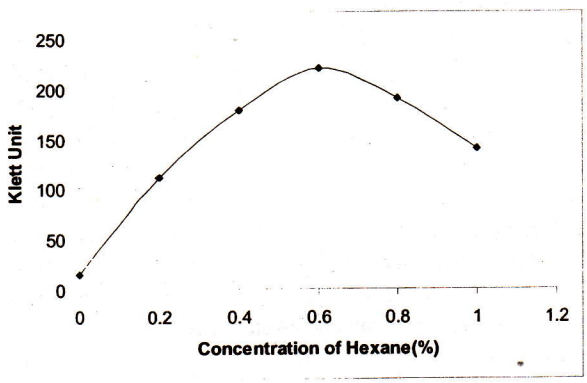

Fig.1. Growth response of *Rhizobium* sp (*Cicer arietinum*) BICC 620 at different concentration of n-hexane.

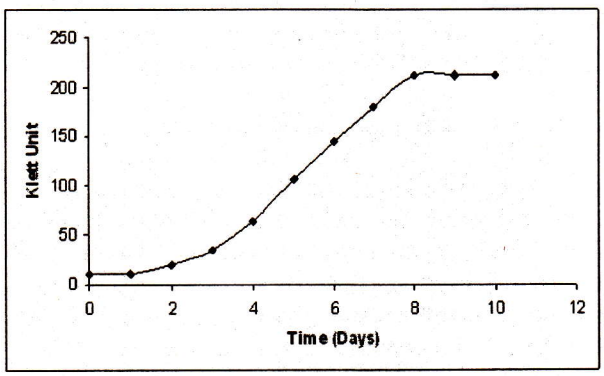

Fig.2. Growth curve of *Rhizobium* sp (*Cicer arietinum*) BICC 620 in Sherwood's synthetic medium containing 0.6% n-hexane as the sole carbon source.

procedures were used to assay the enzymes, Phosphofructokinase (PFK) and fructose bisphosphate aldolase (FBPA)[13], glyceraldehyde-3-phosphate dehydrogenase (Gl3PD)[14], 'ED enzymes' as the combined activity of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase[15], 6 phosphoglucanate dehydrogenase (6PGD)[16], isocitrate dehydrogenase (ICDH)[17], malate dehydrogenase (MDH)[18], and fructose-1,6-bisphosphatase[19]. Assay for each enzyme was run in duplicate, and the values obtained were averaged. Protein was determined by the method of Lowry *et al*[20].

Results and Discussion

The fast growing *Rhizobium* sp. (*Cicer arietinum*) BICC 620 cells can grow at different concentration of hexane with a maximum growth at 0.6% which supported about

215 Klett unit of growth, then a declining trend at further higher concentration (Fig.1). The strain grew at a very slow rate and reach to stationary phase at about eighth day through a mid log phase at 6th day preceded by more than one day of lag phase (Fig.2). CFE of such hexane grown mid-log phase culture cells showed insignificant or no activities of glycolytic enzymes compared to glucose grown cells when assayed for key enzymes of the Embden Meyerhof Parnas- (PFK, FBPA), Entner Doudoroff- (ED-enzymes), and pentose phosphate- (6-PGD) pathways (Table 1). Although very less but definite presence of activity of Gl3PD (9.2 n mole of substrate consumed per min^{-1} per mg^{-1} protein) was observed from the cells. The only EMP pathway enzyme, Gl3PD was detected from the CFE of such hexane grown cells indicate its involvement with other intermediary metabolic pathways as was found earlier in carbon starved cells[6]. Tri carboxylic acid (TCA) cycle enzymes, viz., MDH and ICDH were also present (Table 1), however, levels of these enzymes were much lower than those of glucose-, mannitol- or even alcohol grown aerobic culture of cells[21,22]. Both ME and FBPase, the enzymes for gluconeogenesis, were present at significant level, which were 39.1 and 41.1, respectively of their specific activities (Table 1). Isocitrate lyase (ICL), the enzyme responsible for bypassing the TCA cycle, a key enzyme for glyoxylate pathway, was also detected from the CFE of hexane grown cells. The TCA cycle enzymes although present at a level almost five fold lower than in hexose grown aerobic cells[21] and nearly half of ethanol or propanol grown cells and equal to butanol grown cells[22]. This comparatively lower level of specific activity of TCA cycle enzymes indicate a very slower rate of generation of ATP , which is also supported by a very slow rate of growth during growth on hexane (Fig.2). As the compound is not supported the growth of the organism well, it can be compared with a situation of carbon famine. Under such situation, organisms normally do not follow high rate of catabolism through glycolytic or TCA or whatever other cycle, rather they also metabolize in a reverse direction through gluconeogenesis to conserve cells available storage and maintained a balance between anabolism and catabolism[7,23]. Detection of substantial activity of FBPase and ME, which are involved in gluconeogenesis indicate a strategy of hexane grown cells in the same line (Table 1).

The flexible and versatile life style of rhizobia, their ability to survive under stressed environmental conditions make them more pertinent in this aspect of utilizing hexane and other soil aromatic components. Recently several studies have pointed out the importance of rhizosphere in decontamination and recycling of pollutants[24]. Studies showing the horizontal transfer of plasmid pJP4, bearing genes for mercury resistance and 2,4-D degradation in rhizobia, mobilization in *R.trifolii* and subsequent co-metabolism of herbicide 2,4-D in soil[25], and enhancement of microbial PCB degradation in soil in presence of a variety of individual chemicals that are plant compounds[26], may indicate toward an existing role of rhizobia in environmental maintenance, their own survival, and a possibility of using them in future.

References

1. Hardy RWF and Havelka UK 1975, Nitrogen fixation research: A key to world food. *Science.* **188** 633-643.
2. Sahgal M and Johri BN 2003, The changing face of rhizobial systematics. *Curr. Sci.* **84** 43-48
3. Glenn AR, McKay IA, Arwas R and Dilworth MJ, 1984, Sugar metabolism and the symbiotic properties of carbohydrate mutant of *Rhizobium leguminosarum*. *J.Gen. Microbiol.* **130** 239-245.
4. Stowers MD 1985, Carbon metabolism in *Rhizobium* species. *Annu. Rev. Microbiol.* **39** 89-108
5. Mandal NC and Chakrabartty PK 1999, Enzymes of carbohydrate metabolism in root nodule bacteria during growth on acetate. *J. Basic Microbiol.* **39** 253-256.
6. Mandal N C 1992, Carbon metabolism in *Rhizobium* sp. (*Cicer arietinum*). Ph.D. Thesis submitted to the Calcutta University.
7. Mandal NC and Chakrabartty P K 1992, Carbohydrate metabolic enzymes of *Rhizobium* during carbon starvation. *Indian J. Exp. Biol.* **30** 804-807.
10. Vincent J M 1970, *A manual for practical study of the root nodule bacteria. I B P Handbook No.15*, (Blackwell Scientific Publications, Oxford).
11. Sherwood M T 1970, Improved synthetic medium for the growth of *Rhizobium*. *J. Appl. Bacteriol.* **33** 708-713.
12. Mandal N C and Chakrabartty P K 1993, Succinate mediated catabolite repression of enzymes of glucose metabolism in root-nodule bacteria. *Curr. Microbiol.* **26** 247-251.
13. Heath H E and Gaudy E T 1978, Relationship between catabolism of glycerol and metabolism of hexose phosphate derivatives by *Pseudomonas aeruginosa*. *J. Bacteriol.* **136** 638-646.
14. Tiwari N P and Campbell J J R 1969, Enzymatic control of the metabolic activity of *Pseudomons aeruginosa* grown in glucose or succinate media. *Biochim. Biophys. Acta* **192** 395-401.

15. Lessie T G, Vander Wyk J C 1972, Multiple forms of *Pseudomonas multivorans* glucose-6-phosphate and 6-phosphogluconate dehydrogenases: differences in size, pyridine nucleotide specificity, and susceptibility to inhibition of adenosine 5' triphosphate. *J. Bacteriol.* **110** 1107-1117.
16. Tigerstrom M V and Campbell J J R 1966, The tricarboxylic acid cycle, the glyoxylate cycle and the enzymes of glucose oxidation in *Pseudomonas aeruginosa. Can. J. Microbiol.* **12** 1015-1022.
17. Khouw B T and McCurdy H D 1969, Tricarboxylic acid cycle enzymes and morphogenesis in *Blastocladiella emersonii. J. Bacteriol.s* **99** 97-205.
18. Englard S and Siegel L 1969, Mitochondral L-malate dehydrogenase of beef extract. *Methods Enzymol.* **13** 99-106.
19. Stowers M D. and Elkan G H 1983, The transport and metabolism of glucose in cowpea rhizobia. *Can. J. Microbiol.* **29** 398-406.
20. Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951, Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193** 266-275.
21. Mandal N C and Chakrabartty P K 1989, Enzymes of carbohydrate metabolism in fast-growing Rhizobium grown on hexoses or succinate. *Indian J. Biochem. Biophys.* **26** 120-122.
22. Mandal N C and Chakrabartty P K 1997, Alcohol metabolism in free-living *Rhizobium* sp. And *Bradyrhizobium* sp., *Indian J. Exp. Biol.* **35** 401-404.
23. Bakshi D, Mandal V and Mandal N C 2006, Change of carbon metabolic activity of *Rhizobium* under carbon starvation. *J. Plant Biochem. Biotechnol.* **15** 67-69.
24. Simonich SL and Hites RA 1994, Importance of vegetation in removing polycyclic aromatic hydrocarbons from atmosphere, *Nature* **370** 49-51.
25. Feng L., Rolfe BG, Gartner E and Zwieten-Kennedy I R 1994, Biodegradation and plant protection from the herbicide 2,4-D by plant microbial association in cotton production system. *Acta Biotechnol.* **14** 119-129.
26. Donelly PK, Hedge RS and Fletcher JS 1994, Growth of PCB degrading bacteria on compounds from photosynthetic plants. *Chemosphere* **28** 981-988.