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ENZYMATIC DIFFERENTIATION OF *AZOTOBACTER* AND *AZOMONAS*

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ACCORDING to the ninth edition of *Bergey's manual of systematic bacteriology*[1], the family Azotobacteraceae consists of two genera, *Azotobacter* and *Azomonas*. Members of the two genera show strong immunological cross-reaction and high rRNA cistron homology. Cyst formation by *Azotobacter* and DNA base composition differentiate members of the two genera. The grouping is further supported by bacteriophage typing[2]. It is, however, not known if there is any enzymatic basis for this separation. Previous investigations reported the operation of the Entner–Duodoroff (ED) pathway for glucose oxidation in *Azotobacter*[3–5]. Enzymes of the Embden–Meyerhof–Parnas (EMP) pathway and pentose phosphate (PP) pathway were also demonstrated in *Azotobacter vinelandii*[6]. The present investigation attempts to make a comparative study of the key enzymes involved in the different pathways of carbohydrate metabolism.

Azotobacter chroococcum BI_1, *Azotobacter chroococcum* BI_2 *Azotobacter vinelandii* Ka were isolated in our laboratory from soil and identified according to the Bergey's manual[1]. *Azotobacter vinelandii* 2821, *Azomonas agilis* 2819 and *Azomonas macrocytogenes* 2454 were procured from the National Collection of Industrial Microorganism (NCIM), Pune, India. The organisms were maintained on slants of Burk's nitrogen-free medium[7] between experiments. The cells were grown in nutrient broth (Hi-Media, Bombay) supplemented with 0.5% sucrose as carbon source for 16 h, when the cells reached late log phase of growth. The cells were harvested by centrifugation at 10,000 *g* in a Sorvall centrifuge. Cell-free extracts were prepared by sonication of cells for a total period of 3 min and the extracts were clarified by centrifugation at 30,000 *g* for 15 min. The supernatant was used for the enzyme assays. 6-Phosphogluconate dehydrogenase (6PGD)[8], ED enzymes (combined activities of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase)[9], 6-phosphofructokinase (PFK) and fructose diphosphate aldolase (FDA)[10], glyceraldehyde-3-phosphate dehydrogenase (GL3PD)[11] and isocitrate dehydrogenase (ISDH)[12] were assayed according to published procedures. Protein was determined in cell-free extracts according to Lowry *et al*[13].

From a survey of the enzymes of the ED, EMP, PP and tricarboxylic acid (TCA) cycle pathways (table 1), an operational ED pathway was detected in all the strains, in confirmation of the previous findings[3–6]. However, the specific activities of the ED enzymes were several-fold higher in the cell-free extracts of the strains of *Azomonas* than in those of the strains of *Azotobacter* under identical conditions of assay. The reason for the higher specific activities of ED enzymes in strains of *Azomonas* is not clear. Small differences in the specific activities of carbon metabolism enzymes have been noted in *Azospirillum brasilense* wild-type strain and its mutants[14], while significant differences in the activities of ED enzymes were noted in strains of *Rhizobium* and *Bradyrhizobium*[15]. Considerable differences in specific activities of ED enzymes in the strains of *Azotobacter* and *Azomonas* would, as such, lend further support to this separation at the generic level. The presence of high levels of PFK, a key enzyme of the EMP pathway, in many strains (table 1) suggests an operational EMP pathway in them, though the activity of FDA, another key enzyme of the EMP pathway, is comparatively low in all the strains of *Azotobacter* studied. This would imply that EMP pathway operates at a lower efficiency in *Azotobacter* than in strains of *Azomonas*. The activity of GL3PD, an enzyme involved in the EMP, ED and PP pathways, was high in all the strains. The high activity of ISDH, a TCA cycle enzyme, in all the

Table 1 *Specific activities of key enzymes of carbohydrate metabolism in cell-free extracts of strains of Azotobacter and Azomonas*

Strain	ED enzymes	6PGD	PFK	FDA	GL3PD	ISDH
Azotobacter chroococcum BI_1	3	27	30	5	375	3143
Azotobacter chroococcum BI_2	3	6	2	9	181	1319
Azotobacter vinelandii 2821	4	354	32	5	699	3055
Azotobacter vinelandii Ka	5	21	96	8	429	295
Azomonas agilis 2819	127	195	7	45	601	1179
Azomonas macrocytogenes 2454	27	40	107	25	38	845

Specific activities expressed as nmol of product formed per minute per mg of protein.

strains studied suggests an operational TCA cycle in all the strains. Activity of NADP-6-phosphogluconate dehydrogenase, the key enzyme of the PP pathway, was also detected in all the strains.

On the basis of the activity of the ED enzymes and of FDA it is possible to distinguish between the strains of *Azotobacter* and *Azomonas*. This separation based on enzymatic determination is consistent with the classification of De Smedt *et al*[16], which is based on rRNA cistron homology, for these strains.

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VISCOMETRIC STUDIES ON THE EFFECT OF NEAR-UV LIGHT IRRADIATION ON DNA IN PRESENCE OF CHLORPROMAZINE

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CHLORPROMAZINE (CPZ), a phenothiazine drug, is now widely used as an antidepressant tranquilizer. The drug is highly photosensitive. It has some effects on patients who use it continuously[1,2]. Dark incubation of human red blood cell (RBC) with this drug (concentration 3×10^{-4} M) causes haemolysis of the RBC[3]. Near-UV light (365 nm) irradiation of RBC in the presence of relatively low concentration of the drug causes extensive haemolysis[3]. Photomutagenesis in presence of CPZ has been reported in Chinese hamster cells[4] and in *Salmonella typhimurium*[5]. Photoinduced inactivation of adenovirus[6] and bacteriophage[7] has also been observed. In this paper we report the results of viscometric studies on the effect of near-UV light (365 nm) irradiation on DNA in presence of CPZ.

CPZ, as CPZ hydrochloride (made in the USSR), was obtained from Sun Pharmaceutical Industries, Vapi, Gujarat, India. A stock solution of CPZ, 1 mg/ml in 0.01 M NaCl, was freshly prepared before each experiment. The absorption spectrum of CPZ solution (19 μg/ml) shows two absorption maxima, one at $\lambda = 255$ nm, with molar extinction coefficient