Aldolase and dehydrogenase activities in *Spirillum bengal*

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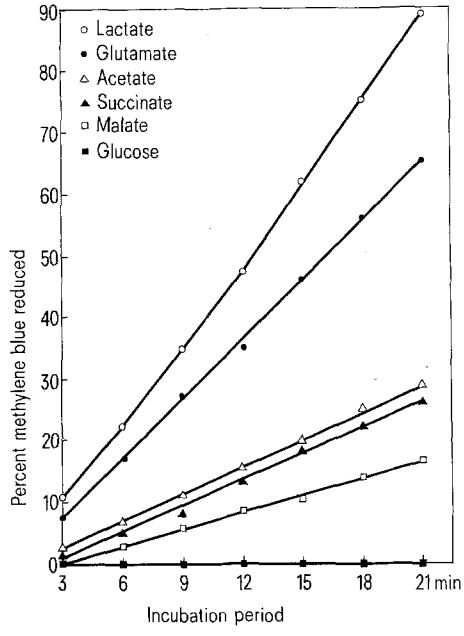
Summary. Spirillum bengal is unable to grow on sugars, but can utilize different organic acids as carbon sources. Dehydrogenase activities were tested with different substrates and were found highest with lactate, glutamate, acetate, succinate and malate. A low aldolase activity was also detectable.

In an earlier study[1], *Spirillum bengal,* isolated from a fresh water pond in West Bengal, was described and its growth physiology was studied. *Spirillum bengal* (strain ATCC 27641) is unable to utilize any sugars as carbon source for growth, but it can grow on organic acids, including tricarbonic acid cycle intermediates. In our previous identification, the organism was named *Aquaspirillum bengal,* but according to the 7th edition of Bergey's Manual, the genus *Aquaspirillum* forms part of the genus *Spirillum.* In this communication we describe an analysis on dehydrogenase and aldolase activities in *S. bengal.*

Materials and methods. The capacity of the spirilla to transfer electrons from lactate, glutamate, acetate, succinate, malate and glucose to methylene blue was tested by the Thunberg technique[2]. Readings were taken in a Klett-Summerson photo-electric colorimeter and the results were expressed as percent methylene blue reduced at different time intervals. The enzyme fructose-1,6-diphosphate aldolase was assayed by the Sibley and Lehninger method[3] in cell-free extracts. Cells were harvested from 72-h-old cultures by centrifugation at 3000×g for 15 min at 5 °C and the cell mass was washed twice with veronal (0.05 M) or Tris-HCl (0.05 M) buffer pH 8.4. The cell mass was then crushed with double the volume of analytical sand in a prechilled mortar for 10 min and then mixed with veronal buffer, adding 20 ml of buffer per g of cells. The mixture was centrifuged at 16,000×g for 20 min at 0 °C. The supernatant cell-free extract was used for assay of the enzyme. The reaction mixture contained 0.2 M veronal buffer pH 8.6 1 ml, 0.05 M fructose-1,6-diphosphate 0.25 ml, 0.56 M hydrazine sulphate 0.25 ml, bacterial extract 0.25 ml and was incubated in a water bath at 38 °C for 15 min. The reaction was stopped by adding 1 ml trichloroacetic acid. From the clear supernatant 1 ml was pipetted into a colorimeter tube and mixed with 1 ml of 0.75 N NaOH solution. After 10 min, 1 ml of 0.1% dinitrophenylhydrazine solution in 2 N HCl was added and the mixture was incubated for 10 min at 38 °C. Finally, the volume was made to 10 ml with 0.75 N NaOH and the colour developed was measured photometrically at 540 nm.

Results and discussion. Dehydrogenase activities are shown in the figure. The inability of *Spirillum bengal* to utilize glucose was corroborated by lack of activity with this substrate. Among the substrates oxidised, lactate and glutamate showed the highest rate of oxidation, followed by acetate, succinate and malate. During 21 min incubation, 89% and 65% of the methylene blue was reduced with lactate and glutamate, respectively.

The enzyme fructose-1,6-diphosphate aldolase was found to be present in cell-free extracts of the organism. The relative aldolase activity of the Bengal spirillum is comparatively much lower than in the other species tested (table). The presence of a low but definite fructose-1,6-diphosphate aldolase activity in the cells of *S. bengal* was unexpected in view of the inability of the organism to utilize sugars. Either aldolase activity may be significant in this organism for gluconeogenesis[4,5], or the inability to utilize sugars may be due to the lack of other enzymes in the glycolytic pathway, or to the lack of sugar permeases.



Oxidation of substrates by intact cells of *S. bengal,* as measured by methylene-blue reduction in Thunberg tubes. Main tube contained 2 ml 0.02 M substrate, 2 ml 0.067 M phosphate buffer pH 7.0 and 1 ml of washed bacterial suspension (turbidity 3.5 EEL units, see the table), prepared from a 72-h-old peptone broth (1%) culture. The side tube contained 1 ml of methylene-blue solution (0.1 mg/ml) adjusted to pH 7.0. Tubes incubated at 37 °C and methylene-blue reduction was measured colorimetrically at 610 nm against a control which contained all other ingredients except the substrate which was substituted by distilled water.

Relative aldolase activity of extracts of *Spirillum bengal, Rhizobium phaseoli, Escherichia coli* and *Saccharomyces cerevisiae*

Organism	Relative aldolase activity EEL units*/mg protein/min
S. bengal strain ATCC 27641	0.08
*R. phaseoli***	0.48
E. coli strain K12	0.51
*S. cerevisiae****	0.75

* Evans Electroselenium Ltd, England. Portable photoelectric colorimeter. ** Isolated from root nodules of *Phaseolus vulgaris.* *** Isolated from 'tower brand' bakers' yeast manufactured by a local company.

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