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Purification and Characterization of a Ribulose Bisphosphate Carboxylase/Oxygenase from *Hydrogenophaga pseudoflava*

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A ribulose bisphosphate carboxylase/oxygenase (RubisCO) from cells of *Hydrogenophaga pseudoflava* grown on carbon monoxide (CO) was purified 54-fold in 9 steps to better than 95% homogeneity, with a yield of 3.5%. The specific activity was 297 nmol of CO_2 incorporated per min per mg of protein. The molecular weight of the native enzyme was determined to be 505,000. Sodium dodecyl sulfate-gel eletrophoresis revealed at least two nonidentical subunits of molecular weights 51,500 and 14,000. The K_m and V_{max} of CO were 16.4 mM and 777.8 nmol/mg protein/min, respectively, and those of ribulose bisphosphate were 0.07 mM and 436.2 nmol/mg protein/min, respectively. The N-terminal amino acid sequence of large subunit was analyzed and was found to be different from those of other enzymes compared including that of *Acinetobacter* sp. strain JC1. One-dimensional peptide map of *H. pseudoflava* enzyme was different from that of the enzyme from CO-grown cells of *Acinetobacter* sp. JC1.

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Growth on Methanol of a Carboxydobacterium, *Acinetobacter* Sp. Strain JC1 DSM 3803

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Acinetobacter sp. strain JC1 DSM 3803, a carboxydobacterium, was found to grow methylotrophically at the expense of methanol and methylamine, but not of methane, formaldehyde, formate, dimethylamine, or trimethylamine, as the sole source of carbon and energy. The doubling times of the bacterium growing on methanol (0.5%, v/v) and methylamine (0.5%, w/v) at 30°C and pH 6.8 were 4.8 h and 5.7 h, respectively. Cells grown on methanol, however, failed to show typical methanol dehydrogenase and oxidase activities. The cell was found to contain no *c*-type cytochromes. Cells grown on methanol exhibited higher catalase activity than those grown on pyruvate or glucose. The catalase present in the cells also exhibited peroxidase activity. The catalase activity, the growth on methanol of the cell and the oxygen consumption by methanol-grown cells were inhibited strongly by 0.1 mM, 1 mM, and 0.1 mM hydroxylamine, respectively. Formaldehyde dehydrogenase, formaldehyde reductase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase activities were detected from cells grown on methanol. The bacterium grown on methanol was also found to show dihydroxyacetone synthase, dihydroxyacetone kinase, and ribulose bisphosphate carboxylase, but no hydroxypyruvate reductase and very low hexulose-6-phosphate synthase, activities.