Various hydrogenases and perspective metabolic pathways for hydrogen production in *Citrobacter amalonaticus* Y19

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Hydrogenases and metabolic pathways related to hydrogen production were investigated in the recently isolated *Citrobacter almalonaticus* Y19. When glucose was used as a carbon source, several organic acids such as lactate, acetate, formate and butyrate were produced along with ethanol and hydrogen. Specific hydrogen production rates from glucose and formate were estimated to be 0.54 μmol H_2/mg cdw/min and 0.44 H_2 μmol/mg cdw/min, respectively. The cell-free extract of Y19 can generate hydrogen with NADH or reduced methylviologen (MV) as an electron donor and the specific activities were determined as 8.1 nmol H_2/mg protein/min and 0.6 μ mol H_2/mg protein/min, respectively. By an ion exchange chromatography with a DEAE packing, the cell-free extract of Y19 was separated to hydrogenase fraction (H-fraction) and ferredoxin fraction (F-fraction). The H-fraction could produce hydrogen by using! reduced MV at a rate of 3.6 μmol H_2/mg protein/min, but could not from NADH. This result suggests that, in addition to the membrane-bound formate-hydrogen-lyase (FHL) complex, Y19 has a soluble hydrogenase whose activity is dependent on ferredoxin.