

Various Hydrogenases and Formate Dependent Hydrogen Production in *Citrobacter amalonaticus* Y19

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An isolate *Citrobacter amalonaticus* Y19 showed a typical mixed-acid fermentation with lactate and acetate as major end products. Production of hydrogen (H₂) from glucose, formate, and reduced methylviologen (MV) and benzylviologen (BV) by the resting cells of Y19 indicates the presence of formate hydrogen lyase (FHL) activity and other hydrogenases. Study with subcellular fractions of Y19 exhibited that the FHL activity, dependent on soluble formate dehydrogenase activity, was detected in the broken cell extract, but not in the soluble or particulate fraction which are separated by centrifugation at 35,000×g. Hydrogen production in the presence of reduced MV or BV was observed in both the soluble and particulate fractions. Uptake hydrogenase activity was observed in the both fractions when the oxidized form of MV and BV were supplied as electron acceptor. In the soluble fraction, when formate was coupled with oxidized form of MV or BV, hydrogen production activity was recovered. These results indicates that, similar to *E. coli*, the strain Y19 expresses two different hydrogenases, one as the FHL complex and another as membrane-associated enzyme.