Oxygen Sensitivity of Carbon Monoxide-Dependent Hydrogen Production Activity in *Citrobacter* sp.

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A newly isolated *Citrobacter* sp. Y19 catalyzes the CO-dependent H_2 production (biological water-gas shift reaction) by the actions of CO dehydrogenase (CODH) and hydrogenase. Y19 requires O_2 for fast growth but its H_2 production activity is significantly inhibited by O_2. The effect of O_2 on the activities of CODH and hydrogenase was investigated quantitatively in both whole cells and broken cells based on CO-dependent or methyl viologen (MV)-dependent H_2 production and CO-dependent MV reduction. In crude cell extracts, CODH activity was mostly found in the soluble fraction. Inactivation of CODH and hydrogenase activities by O_2 followed the first-order decay kinetics and the dependence of the rate constants on O_2 partial pressure could be expressed by Michaelis-Menten equation. In whole cell, the maximum deactivation rate constants ($k_{d,max}$) of hydrogenase and CODH were quite similar as 0.07 ± 0.03 $min^{-1}$and 0.10 ± 0.04 min^{-1}, respectively. However, the first-order rate constant ($k_{d,max}/K_s$) of CODH (0.25 min^{-1} atm^{-1}) at low O_2 partial pressures was about 3-fold higher than that of hydrogenase since the half-saturation constant (K_s) of CODH was about half of that of hydrogenase. In broken cells, both enzymes became significantly more sensitive to O_2 compared to the unbroken cells and $k_{d,max}/K_s$ increased 37-fold for hydrogenase and 6.7-fold for CODH. When the whole cells were incubated under anaerobic conditions after exposure to air for 1 h, hydrogenase activity was recovered more than 90% in 2 h suggesting the deactivation of hydrogenase by O_2 was reversible. On the contrary, CODH activity was not recovered once deactivated by O_2 and the only way to recover the activity was the synthesis of new CODH. This study indicates that O_2 sensitivity of H_2 production activity of *Citrobacter* sp. Y19 is an important drawback as in other H_2-producing bacteria.

REFERENCES

1. Adams, M. M. W. "The structure and mechanism of iron-hydrogenases." (1990), *Biochim.*

Biophys. Acta. **1020**, 115-145.

2. Adams, M. M. W. and D. O. Hall. "Purification of the membrane-bound hydrogenase of *Escherichia coli.*"(1979), *Bioch. J.* **183**, 11-22.
3. Bonam, D., L. Lehman, G. P. Roberts, and P. W. Ludden. "Regulation of carbon monoxide dehydrogenase and hydrogenase in *Rhodospirillum rubrum*: Effect of CO and oxygen on synthesis and activity." (1989), *J. Bacteriol.* **171(6)**, 3102-3107.
4. Bonam, D., M. C. Mckenna, P. J. Stephens, and P. W. Ludden. "Nickel-deficient carbon monoxide dehydrogenase from *Rhodospirillum rubrum*: *In vivo* and *in vitro* activation by exogenous nickel." (1988), *Proc. Natl. Acad. Sci. USA.* **85**, 31-35.
5. Bonam, D., S. A. Murrell, and P. W. Ludden. "Carbon monoxide dehydrogenase from *Rhodospirillum rubrum.*" (1984), *J. Bacteriol.* **159(2)**, 693-699.
6. Jung, G. Y., J. R. Kim, H. O. Jung, J. Y. Park, and S. Park. "A new chemoheterotrophic bacterium catalyzing water-gas shift reaction." (1999), *Biotechnol. Lett.* **21**, 869-873.
7. Jung, G. Y., J. R. Kim, J. Y. Park, and S Park. "Hydrogen production by a new chemoheterotrophic bacterium *Citrobacter* sp. Y19." (2002), *Int. J. Hydrogen Energy.* **27**, 601-610.