Continuous culture studies on the regulation of PQQ-dependent glucose dehydrogenase in *Acinetobacter calcoaceticus*

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Irrespective of the growth substrate *Acinetobacter calcoaceticus* cells are capable of oxidizing glucose. Addition of glucose to the growth medium does not enhance this capacity. One single enzyme, namely glucose dehydrogenase, is responsible for this oxidation. Addition of glucose to cultures of *A. calcoaceticus* results in its quantitative conversion to gluconic acid (De Bont et al., 1984). Glucose dehydrogenase (GDH) is a membrane-bound periplasmic enzyme. It contains PQQ as a prosthetic group which donates its electrons directly to the electron transport chain.

Since *A. calcoaceticus* is a very versatile organism, capable of aerobic growth on at least 80 organic compounds, it is rather surprising that GDH is synthesized constitutively. Moreover, since *A. calcoaceticus* LMD 79.41 is not capable of growth on glucose of gluconate, it is rather peculiar that GDH is synthesized at all.

In order to gain more information on the possible role of this enzyme in energy metabolism of *A. calcoaceticus* we performed a continuous culture study on the physiology of this organism with special attention to the regulation of GDH synthesis.

Cells were cultured in mineral medium with acetate as a sole carbon and energy source, on which *A. calcoaceticus* grows very rapidly (μ_{max} of 1.26 h^{-1}). Upon decreasing the dilution rate from 0.55 h^{-1}, the influence of the maintenance energy became already apparent at dilution rates below 0.2 h^{-1}. From a Pirt plot values for m_e and Y^{max} of 3.8 mmol acetate·g cells^{-1}·h^{-1} and 26 g cells·mol acetate^{-1}, respectively, could be calculated. This cell yield is rather low but comparable to that of other oxidase-negative (cytochrome *c*-lacking) bacteria.

GDH activity was determined in cell-free extracts, using a PES/DCPIP spectrophotometric assay. The levels of this enzyme increased 5-10-fold with decreasing growth rate. Various growth conditions were tested at a dilution rate of 0.15 h^{-1} with acetate-grown cultures, including variations in culture pH and temperature, nitrogen limitation and oxygen limitation. Of these only oxygen limitation significantly affected GDH synthesis. During oxygen-limited growth the levels of GDH decreased 10-20-fold. These results are in agreement with the observation that under all growth conditions, except under oxygen limitation, inclusion of glucose into the growth medium resulted in the production of gluconate.

DE BONT, J. A. M., DOKTER, P., VAN SCHIE, B. J., VAN DIJKEN, J. P., FRANK JZN, J., DUINE, J. and KUENEN, J. G. 1984. Role of quinoprotein glucose-dehydrogenase in gluconic acid production by *Acinetobacter calcoaceticus*. — Antonie van Leeuwenhoek **50**: 76–77.