Open access • Journal Article • DOI:10.1007/BF00404542

Further Evidence for Aerobic Denitrification by Thiosphaera-Pantotropha

Lesley A. Robertson, J.G. Kuenen

Institutions: Delft University of Technology

Published on: 01 Jan 1985 - Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology (Kluwer Academic Publishers)

Topics: Aerobic denitrification and Denitrification

Energization of solute transport by PQQ-dependent glucose dehydrogenase in membrane vesicles of *Acinetobacter* species

J. T. PRONK, B. J. VAN SCHIE, J. P. VAN DIJKEN and J. G. KUENEN

Laboratory of Microbiology, Delft University of Technology, Julianalaan 67A, 2628 BC Delft, The Netherlands

Most *Acinetobacter* strains can not grow on glucose or gluconic acid as a sole carbon source. However, when glucose is added to the growth medium, it is quantitatively oxidized to gluconic acid. The enzyme responsible for this reaction is a membrane-bound glucose dehydrogenase (GDH, EC 1.1.99.17) which contains PQQ as a prosthetic group.

GDH synthesis is constitutive in *A. calcoaceticus*. Strains of *A. lwoffi*, on the other hand, are unable to oxidize glucose but nevertheless contain apo-GDH under all growth conditions. Addition of the prosthetic group PQQ is required to restore GDH activity in *A. lwoffi* (Van Schie et al., 1984).

In order to study the functional coupling of GDH to the respiratory chain we developed a procedure for the isolation of membrane vesicles from bacteria grown under well-defined conditions. Cells were collected at 4 °C from an acetate-limited continuous culture. The method for the isolation of membrane vesicles was a modification of the procedure described for *Pseudomonas aeruginosa* by Stinnett et al. (1973). Membrane vesicles prepared from carbon-limited chemostat cultures of *A. calcoaceticus* LMD 79.41 and *A. lwoffi* LMD 83.25 exhibited active transport of alanine energized by GDH. In case of *A. lwoffi* both oxidation of glucose and active transport energized by glucose oxidation were dependent on the presence of PQQ. The rate of alanine uptake when energized with GDH was comparable with the rate of uptake energized by the artificial electron donor system ascorbate phenazine methosulphate (Table 1).

Table 1. Initial rates of alanine uptake by membrane vesicles of *Acinetobacter calcoaceticus* and *A. lwoffi*

Vesicle preparation	Electron donor	Initial rate of alanine uptake (nmol · min^{-1} · mg protein^{-1})
A. calcoaceticus	none	0.03
A. calcoaceticus	PMS + ascorbate	1.35
A. calcoaceticus	glucose	1.20
A. lwoffi	none	0.01
A. lwoffi	PMS + ascorbate	0.15
A. lwoffi	glucose	0.01
A. lwoffi	glucose + PQQ	0.11

STINNETT, J. D., GUYMON, L. F. and EAGON, R. G. 1973. A novel technique for the preparation of transport-active membrane vesicles from *Pseudomonas aeruginosa*: observations on gluconate transport. — Biochem. Biophys. Res. Commun. **52**: 284–290.

VAN SCHIE, B. J., VAN DIJKEN, J. P. and KUENEN, J. G. 1984. Non-coordinated synthesis of glucose dehydrogenase and its prosthetic group PQQ in *Acinetobacter* and *Pseudomonas* species. — FEMS Microbiol. Lett. **24**: 133–138.