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Denitrifying *Blastobacter* sp. from marine intertidal sediments

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Trying to cultivate a mixed population of denitrifiers from tidal sediment, we observed a pronounced dependence on the presence of surface-rich carrier-material (e.g. glass beads or glowed seasand). Glucose could be used as electron-donor, but the time gap between disappearance of glucose and start of nitrogen-formation indicated that glucose was not the immediate carbon-substrate.

Five pure strains of denitrifiers were isolated from a wadden sediment mesocosm which we have been maintained in a glass house since 5 years. One of the strains (strain 33), which degraded lactate and acetate under denitrifying conditions, but not glucose and glycolate, was identified by NCIMB (National Collection of Industrial and Marine Bacteria, Aberdeen, GB) as *Blastobacter* sp. (HIRSCH and MÜLLER 1985). *Blastobacter* is Gram negative, ovoid or rod-shaped. It forms large amounts of filamentous slime, which among others obviously facilitates attachment to the carrier-material. It required a carrier for growth just as observed for the mixed populations and was halophil. It did not grow in a freshwater medium. It could be cultivated with lactate or acetate and with nitrate or nitrite under anaerobic denitrifying and under aerobic conditions. Aerobic growth rate in the presence of nitrate was four times faster then under anaerobic conditions (nitrite not yet tested). No lag-phase could be observed for growth or substrate degradation when switching from aerobic to anaerobic denitrifying conditions. Fig. 1 shows anaerobic acetate-dependent growth.

Cell-free extracts were prepared by vigorous shaking with glass beads and by grinding cell-mass in a mortar in liquid nitrogen. The 9000 x g-supernatant catalyzed reduction of nitrite to N_2O by hydrosulphite in presence of catalytic amounts of benzyl viologen. K_M (nitrite) was 0.36 mmoles/l (Fig. 2). The cell free enzyme activity was far below the nitrite reduction activity of whole cells. Nitrite reductase activity was at least ten-fold higher in nitrite-grown cells. In this case the observed reductase activity corresponded approximately to the nitrite reduction catalyzed by whole cells. High nitrite concentrations of 150 mmoles/l produced a marked lag phase (up to several weeks), but in the exponential phase growth rate was similar as in the presence of the same concentration of nitrate.

Blastobacter sp. is the first marine denitrifying *Blastobacter*-strain described up to now. It exhibits all properties to be expected for a marine sediment-denitrifier: attachment to sediment-particles, facultative anaerobe, constitutive enzymatic components of denitrification, no growth in freshwater. Nevertheless it is not yet clear, whether *Blastobacter* "33" is a dominant denitrifier in wadden sediments or whether it became dominant only under our enrichment conditions.

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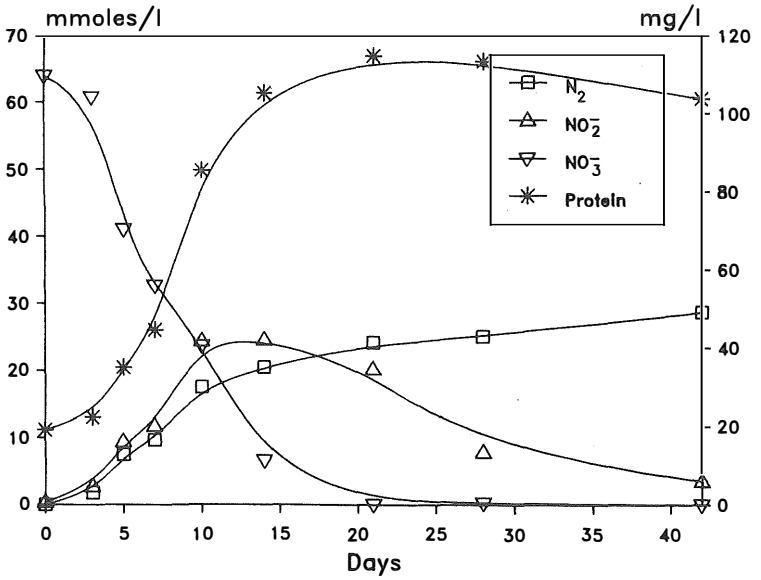

Fig. 1. Growth of *Blastobacter* (followed by protein assay, right ordinate) in anaerobic acetate medium. Nitrate decrease, nitrogen formation and temporary nitrite formation are shown (left ordinate).

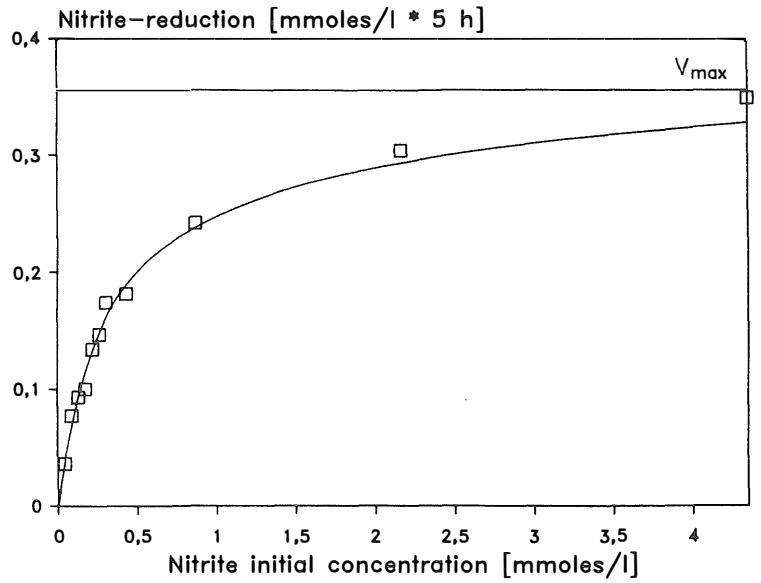

Fig. 2. Substrate saturation curve of nitrite reductase raw extract for nitrite. Curve computed after direct linear plot method. K_m (median value) = 0.364 mmoles/l.

Reference

HIRSCH, P. and M. MÜLLER, 1985. *Blastobacter aggregatus* sp. nov., *Blastobacter capsulatus* sp. nov., and *Blastobacter denitrificans* sp. nov., new budding bacteria from freshwater habitats. System. Appl. Microbiol. 6, 281-286.