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Hypotheses on dissimilatory nitrate reduction pathways in *Bacillus azotoformans*

Introduction

The genome of the phenotypically denitrifying type strain of *Bacillus azotoformans* LMG 9581^T was sequenced and the pathways for dissimilatory nitrate reduction were reconstructed (Heylen & Keltjens, 2012). Genome analyses suggest that denitrification proceeds in the periplasmic space and in an analogous fashion as in Gram-negative organisms, yet with the participation of proteins that tend to be membrane-bound or membrane-associated. A considerable degree of functional redundancy was observed. **In addition to the already characterized menaquinol/cyt *c*-dependent nitric oxide reduction (Suharti et al., 2001;Suharti et al., 2004) of which the encoding genes could be identified now, evidence for another novel nitric oxide reductase was found.** Also, our analyses confirm earlier findings on branched electron transfer with both menaquinol and cytochrome *c* as reductants. **A new organization of the periplasmatic nitrate reductase NAP system** was also described. And quite unexpectedly, *B. azotoformans* LMG 9581^T has the disposal of **two parallel pathways for nitrite reduction** hypothetically enabling a life style as a denitrifier and as an ammonifying bacterium. Now, however, more questions have been raised on the actual pathways used by *B. azotoformans* than answers found. Here, we show an overview of the enzymes involved in dissimilatory nitrate reduction (DNR) pathways deduced from the gene inventories and present some research hypotheses.

Topological model of denitrification enzymes and other associated electron-transfer

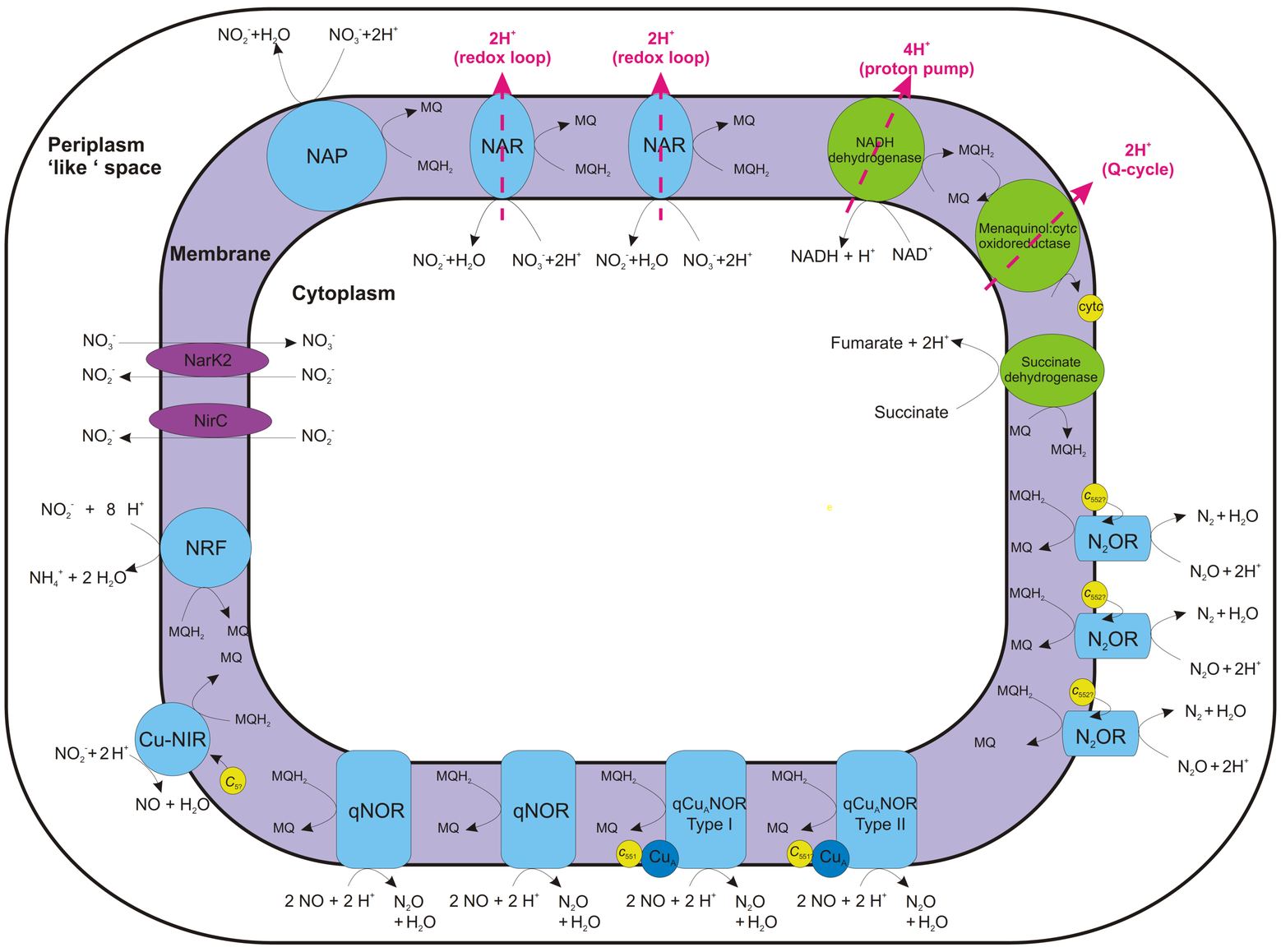

Figure 1. Menaquinol (MQH_2) donates electrons to a menaquinol:cytochrome c oxidoreductase (related to bc_1/b_6f), NAR, NIR, NRF, NOR and N_2OR. Reduction of all the membrane-bound *c*-type cytochromes is coupled with menaquinol oxidation mediated by the menaquinol:cytochrome *c* oxidoreductase. Membrane-bound *c*-type cytochromes subsequently donate electron to their corresponding enzymes.
The location of the substrate-binding sites are hypothesized to be similar to other bacteria. Enzymes involved in respiratory nitrate reduction are depicted in blue. Nitrate/nitrite transport system are in purple. Menaquinol:cytochrome *c* oxidoreductase (related to bc_1/b_6f), NADH dehydrogenase and succinate dehydrogenase are in green. Charge displacements contributing to the proton motive force are in pink. Cytochrome *c* lipoproteins are in yellow.
Deduced from genome data or taken from Suharti & De Vries (2005).

HYPOTHESIS 1: Various scenarios are non-redundant

Figure 2. All hypothetically possible DNR pathways are depicted. These genes can be expressed in different contexts or may have slightly different structural roles. This « genetic redundancy » at the functional gene levels might reflect the ecological strategy of *B. azotoformans* in the soil matrix (cfr rRNA operon copy number as described by Klappenbach *et al*, 2000). We here assume that the organism only utilizes one functional gene per reduction step.
NARGs have 74.9% AA sequence identity; qNORs only 38%; N_2OR between 76.5 – 83.1%.

HYPOTHESIS 2: DNRA is only for energy conservation

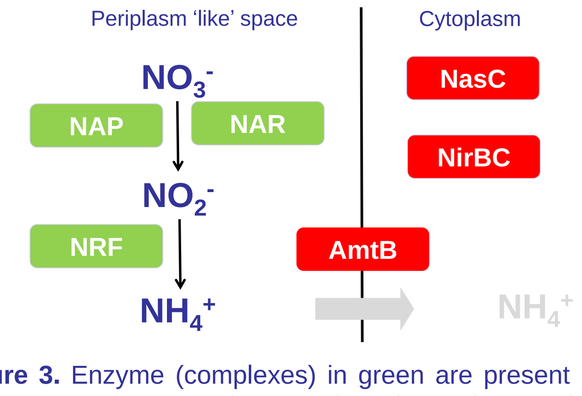

Figure 3. Enzyme (complexes) in green are present in the genome of *B. azotoformans*, in red are absent. This would make *B. azotoformans* dependent on ammonium as the nitrogen source. Therefore, the DNRA, if expressed, should be involved in energy conservation**.** Because of the difference in energy yield per mole nitrate and carbon, it has been argued that denitrification is more favorable under carbon limitation, and DNRA is more attractive under electron acceptor limitation.

HYPOTHESIS 3: N_2O emission is not only linked to denitrification

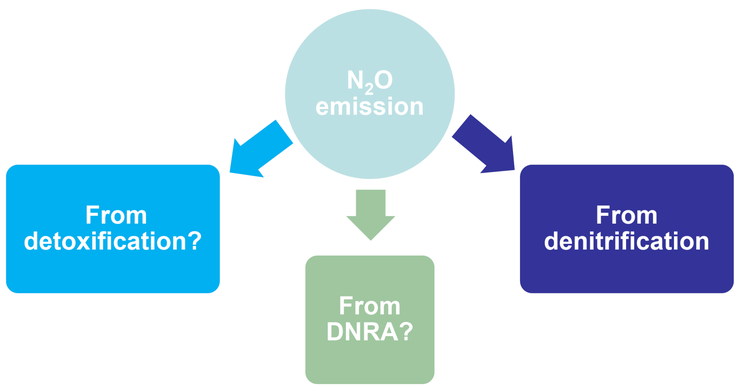

Figure 4. Nitrous oxide gas can be emitted via various pathways all including a NOR type enzyme. So far, it is unclear if any of the NOR types are preferentially involved in any of the three pathways known to emit nitrous oxide gas. However, the presence of four genes for this function does imply vigorous N_2O production.

Functional « redundancy » leads to metabolic versatility

B. azotoformans is a versatile soil bacterium adapted to living in the soil matrix. Soil provides an array of microenvironments that can vary considerably with regard to resource availability.
It is possible that *B. azotoformans* has two parallel pathways for nitrite reduction enabling a **dual life style as a denitrifier and as a DNRA bacterium.** This « redundancy » on the pathway level as well as on the functional gene level may broaden the adaptive ability of *B. azotoformans* and may be (in part) at the basis of its successful lifestyle.

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