Genomic View of Energy Metabolism in *Ralstonia eutropha* H16

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Key Words

Energy metabolism • *Ralstonia eutropha* • Lithoautotrophy • Hydrogen oxidation • Denitrification • Electron transport pathways

Abstract

Ralstonia eutropha is a strictly respiratory facultative lithoautotrophic β -proteobacterium. In the absence of organic substrates, H_2 and CO_2 are used as sole sources of energy and carbon. In the absence of oxygen, the organism can respire by denitrification. The recent determination of the complete genome sequence of strain H16 provides the opportunity to reconcile the results of previous physiological and biochemical studies in light of the coding capacity. These analyses revealed genes for several isoenzymes, permit assignment of well-known physiological functions to previously unidentified genes, and suggest the presence of unknown components of energy metabolism. The respiratory chain is fueled by two NADH dehydrogenases, two uptake hydrogenases and at least three formate dehydrogenases. The presence of genes for five quinol oxidases and three cytochrome oxidases indicates that the aerobic respiration chain adapts to varying concentrations of dioxygen. Several additional components may act in balancing or dissipation of redox energy. Paralogous sets of nitrate reductase and nitric oxide reductase genes result in enzymatic redundancy for denitrification.

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Introduction

Ralstonia eutropha is a strictly respiratory facultative lithoautotrophic soil and freshwater bacterium of the β -subgroup of proteobacteria [Aragno and Schlegel, 1992]. Strain H16 is one of the best-studied model organisms for lithoautotrophic growth on molecular hydrogen and carbon dioxide [Friedrich and Schwartz, 1993]. Under conditions of anoxia, the bacterium can switch to denitrification by using nitrate as alternative electron acceptor [Römermann and Friedrich, 1985]. Organic carbon and energy sources for heterotrophic growth include TCA cycle intermediates, sugar acids, fatty acids, amino acids, alcohols, and aromatic compounds, while utilization of sugars is restricted to fructose and *N*-acetylglucosamine [Johnson and Stanier, 1971; Kersters and De Ley, 1984]. Fructose is catabolized via the Entner-Doudoroff pathway [Gottschalk et al., 1964]. Activities of the key enzymes of the Embden-Meyerhoff-Parnas and the oxidative pentose phosphate pathways, phosphofructokinase and 6-phosphogluconate dehydrogenase, were not detected.

Recently, the complete nucleotide sequence of strain H16 was determined [Pohlmann et al., 2006; Schwartz et al., 2003]. The genome consists of three circular replicons: chromosome 1 (4,052,032 bp), chromosome 2 (2,912,490 bp), and megaplasmid pHG1 (452,156 bp). Both the genomic size of 7.42 Mbp and a total of 6,626 genes lie within the upper range of proteobacterial ge-

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Fig. 1. Schematic representation of energy metabolism in *R. eutropha* H16. Replicons are indicated by numbers: 1 = chromosome 1; 2 = chromosome 2; 3 = megaplasmid pHG1. CBB = Calvin-Benson-Bassham pathway; TCA = tricarboxylic acid cycle; ED = Entner-Doudoroff pathway; UQ = ubiquinone; MQ = menaquinone.

nomes. Knowledge of the complete genome sequence provides the opportunity to reconcile the results of previous physiological and biochemical studies in light of the metabolic capacity of this strain, as anticipated from the genome sequence. This survey focuses on the energy metabolism of *R. eutropha* H16. Attempts are made to dissect the constituents of the respiratory chain formed under aerobic chemoorganotrophic, lithoautotrophic, and denitrifying growth conditions.

Lithotrophic Growth

In the lithotrophic growth mode, molecular hydrogen is split into electrons and protons by two hydrogenases: a membrane-bound enzyme facing the periplasmic side

(membrane-bound hydrogenase, MBH), and a cytoplasmic enzyme (soluble hydrogenase, SH) [Burgdorf et al., 2005]. A third non-energy-conserving hydrogenase (regulatory hydrogenase, RH) functions as hydrogen sensor and interacts with a two-component regulatory system for hydrogen-responsive activation of MBH and SH gene expression [Lenz et al., 2002]. The three hydrogenases have been the subject of comprehensive reviews [Burgdorf et al., 2005; Friedrich and Schwartz, 1993; Lenz et al., 2002]. In the present review, certain biochemical and genetic properties of the MBH and the SH are revisited in the context of energy metabolism. Both enzymes are included in the schematic overview given in figure 1 that illustrates the various electron transport pathways of *R. eutropha* H16.

Table 1. Midpoint potentials (in mV) of membrane-associated cytochromes [data were extracted from Kömen et al., 1991a, and Bernhard et al., 1997]

Heme type ^a	Lithoautotrophic growth ^b		Heterotrophic growth ^b
	M ^c	L ^c	L ^c
A	+255	+255	ND
A	+350	+350	ND
A	(+420) ^d	(+420) ^d	ND
B	+5	+5	+10
B	+75	+75	+40
B	+205	+205	+92
B	+300	+300	+166
B	+405	+405	+405
C	+160	+160	+190
C	+270	+270	+267
C	+335	+335	+330

ND = Not determined.

^a For definitions of heme types, see Nomenclature Committee of the International Union of Biochemistry, 1992.

^b Hydrogenase gene expression was derepressed under both growth conditions.

^c Membrane extracts were prepared from cells in the mid-exponential (M) or late-exponential (L) growth phase.

^d The presence of this component is questionable since it was poorly resolved by redox titration.

Biochemical Properties of MBH and SH

The MBH belongs to a large protein family of uptake [NiFe]-hydrogenases present in bacteria and archaea [Vignais and Colbeau, 2004]. The enzyme is a heterotrimer, consisting of a catalytic subunit (HoxG), an electron transfer subunit (HoxK), and a membrane anchor (HoxZ). The HoxKG moiety is oriented towards the periplasmic side of the cytoplasmic membrane. The Ni-Fe center in HoxG is site of hydrogen splitting. Titration of the active center, followed by electron paramagnetic resonance (EPR) spectroscopy, revealed a midpoint potential $E_{m7} = -110$ mV for Ni. HoxK carries two 4Fe-4S clusters with midpoint potentials $E_{m7} = -180$ mV and $E_{m7} = -60$ mV, respectively, and one 3Fe-4S cluster with $E_{m7} = +25$ mV [Knüttel et al., 1994]. Two *b*-type hemes, present in cells grown under hydrogenase-derepressing heterotrophic conditions, could be assigned to HoxZ [Bernhard et al., 1997]. These hemes had midpoint potentials of $E_{m7} = +10$ mV and $E_{m7} = +166$ mV, respectively (table 1), and were absent in the HoxZ-negative mutant HF405. In addition, HF405 lacked a *c*-type heme with a midpoint potential at $E_{m7} = +190$ mV. If this component is linked to hydrogen metabolism remains to be investigated. HF405 produces a

soluble MBH conformer that is inactive in vivo but shows hydrogen-oxidizing activity with phenazine methosulfate as artificial electron acceptor [Bernhard et al., 1996], indicating that HoxZ couples the enzyme to the electron transport chain. HoxZ was found to be in redox equilibrium with the ubiquinone pool ($E_{m7} = +90$ mV) [Bernhard et al., 1997]. Presumably, electrons are shuttled from the active site in HoxG via the three Fe-S clusters in HoxK and both hemes in HoxZ to ubiquinone. For thermodynamic reasons, the high midpoint potential of the 3Fe-4S cluster in HoxK appears to exclude this center from the intramolecular electron pathway. Nevertheless, a comparison with the crystal structure of the [NiFe] hydrogenase from *Desulfovibrio gigas* [Volbeda et al., 1995] suggests that the 3Fe-4S cluster is located halfway between the two 4Fe-4S clusters in HoxK. Thus the biological function of the 3Fe-4S cluster in HoxK remains to be elucidated.

The SH belongs to the so-called bidirectional hydrogenases [Tamagnini et al., 2002; Vignais and Colbeau, 2004] that directly reduce NAD^+ to NADH at the expense of hydrogen. Under conditions of an increased NADH/ NAD^+ ratio, these enzymes are able to catalyze the reverse reaction, thus possibly acting as a redox valve to prevent overreduction of the electron transfer chain. The SH is composed of two functional modules: a hydrogenase heterodimer consisting of HoxH and HoxY, and a diaphorase dimer consisting of HoxF and HoxU [Burgdorf et al., 2005]. HoxH harbors a Ni-Fe-active center which deviates from that of standard hydrogenases and thus may contribute to the tolerance of the enzyme towards dioxygen [Van der Linden et al., 2004]. The electron transfer subunit HoxY contains one iron-sulfur cluster of the 4Fe-4S type and a labile FMN that was suggested to play a role in the mechanism of H_2 cleavage [van der Linden et al., 2004]. The diaphorase module is believed to contain four iron sulfur clusters: one 4Fe-4S cluster and probably two 2Fe-2S clusters in HoxU, and one 4Fe-4S cluster in HoxF. The HoxF subunit contains an additional FMN molecule [van der Linden et al., 2004] and a binding site for the electron acceptor, NAD^+ . A homodimer of an additional protein HoxI, which is bound to HoxY, may provide a binding pocket for NADP^+ [Burgdorf et al., 2005]. Two identical centers, each of the [4Fe-4S] and of the [2Fe-2S] type with midpoint potentials of $E_{m7} = -445$ mV and $E_{m7} = -325$ mV, respectively, have been detected in the SH by EPR spectroscopy [Schneider et al., 1979].

Hydrogenase Genes

The genes for hydrogen oxidation reside on the megaplasmid pHG1 [Eberz et al., 1986; Schwartz et al., 2003].

Genes for the MBH and associated accessory proteins are located on a 21-kb DNA segment. The RH dimer is encoded in the immediate vicinity of that region. The SH and its accessory functions are encoded on a 10-kb segment that is separated from the MBH genes by 59 kb. Within this spacing region, two genes (*PHG064*, *PHG065*)¹ with coding capacity for a fourth uptake [NiFe]-hydrogenase (Hyd4) are present. In addition, several accessory genes (*hypF3*, *hypC2*, *hypD2*, *hypE2*, *hypA3*, *hypB3*) for hydrogenase maturation are located adjacent to *PHG065*. For a review on the function of *hyp* genes, see Blokesch et al. [2002]. The *hyd4* DNA region is characterized by an unusually high G+C content (above 67%) compared to a mean G+C content of 62.3% of pHG1. In fact, the derived proteins appear to be closely related to a putative [NiFe]-hydrogenase from the actinobacterium *Streptomyces avermitilis*. The physiological role of the Hyd4 enzyme, if any, remains obscure since mutant studies showed that *R. eutropha* H16 is unable to grow on hydrogen in the absence of MBH and SH enzymes [Kleihues et al., 2000].

Yet another hydrogenase-like enzyme is encoded on chromosome 1 within an apparent cluster of seven genes (*A2195* to *A2201*). *A2195* encodes a hypothetical protein of unknown function. Another five genes of this cluster are similar to genes (namely *hycB*, *hycC*, *hycD*, *hycE*, and *hycG*) of the multi-subunit hydrogenase 3 of *Escherichia coli* that is part of a non-energy-conserving formate hydrogen lyase (FHL-1) complex [Sawers, 1994]. The product of *A2198* is similar to HyfE, a polytopic membrane protein encoded in the hydrogenase 4 gene region of *E. coli*. However, four strictly conserved cysteine residues that are involved in coordination of the [NiFe] active center in the hydrogenase catalytic subunit HycE of *E. coli* are replaced to serine and glycine, respectively, in *R. eutropha* H16 HycE. Therefore it is unlikely that the *hyc* genes of *R. eutropha* H16 encode a hydrogen-evolving [NiFe]-hydrogenase. Although hydrogen production was observed when heterotrophically grown cells were exposed to anaerobic conditions, this activity was independent of formate and has been assigned to a reversed reaction of the SH enzyme in the presence of excess of NADH [Kuhn et al., 1984]. An FHL activity could not be detected.

¹ Genes without common names are specified by alphanumerical locus tags, according to the published genome annotation. Locus tags Annnn (where n is any digit) denote genes from chromosome 1, locus tags Bnnnn denote genes from chromosome 2, and locus tags PHGnnn denote genes from pHG1.

Sulfur Oxidation Genes

Lithotrophic growth of *R. eutropha* H16 on reduced inorganic sulfur compounds has not been described. However, eight sulfur oxidation (*sox*) genes *soxR*, *-C*, *-D*, *-Y*, *-Z*, *-A*, *-X*, and *-B* are present on chromosome 1. The genes encode an ArsR-type regulator (SoxR), a sulfur dehydrogenase (SoxCD), a sulfur compound chelating/binding complex (SoxYZ), a thiosulfate-oxidizing complex (SoxAX), and a sulfate thiol esterase (SoxB). All of these products are typical components of the lithotrophic sulfur oxidation pathway [Friedrich et al., 2001, 2005]. The gene region also encodes a small cytochrome *c* (*A3570*), a DsbE-like thioredoxin (*A3564*) and a protein without known functional domains (*A3567*). Furthermore, the product of *hcaD* upstream of *soxR* is 42% identical to the sulfide dehydrogenase SoxF of *Paracoccus denitrificans*. However, genes for the thioredoxins SoxS, SoxW, and for the transmembrane protein SoxV are absent in this region. SoxS and SoxV are important for lithotrophic growth of *P. pantotropha* on thiosulfate and appear to act as electron donors and/or activators of one or more components of the Sox enzyme system [Bardischewsky et al., 2006; Orawski et al., 2007]. Attempts to grow *R. eutropha* H16 on thiosulfate as sole energy source were not successful [C.G. Friedrich, pers. commun.]. Therefore, the *sox* genes are either not expressed at all, the lack of SoxS, SoxW, and SoxV obstructs an otherwise intact pathway, or appropriate growth conditions have yet to be discovered.

Autotrophic Growth and One-Carbon Metabolism

Like all proteobacterial chemoautotrophs, *R. eutropha* H16 uses the Calvin-Benson-Bassham (CBB) pathway for assimilation of CO₂ [Bowien and Schlegel, 1981]. A large *cbbc* operon of 13 genes is located on chromosome 2 that encodes the key enzymes of the CBB cycle [Kusian and Bowien, 1997]. The genes for triose phosphate isomerase (*tim*) and a ribose-5-phosphate isomerase (*rpiA*) are located elsewhere on chromosome 1. A second highly homologous *cbbp* operon of 12 genes is present on pHG1. Both operons are controlled by the LysR-type transcriptional regulator CbbR [Bowien and Kusian, 2002] that is encoded upstream of *cbbl* on chromosome 2. A *cbbR* remnant upstream of the *cbbp* operon is inactive due to several short deletions. Autotrophic growth at air concentrations (0.035% [vol/vol]) of CO₂ further requires a β -carbonic anhydrase [Kusian et al., 2002] that is encoded on chromosome 1 (*can1*). The physiological relevance

of a paralogous β -carbonic anhydrase and an α -carbonic anhydrase, both encoded on chromosome 2 by *can2* and *caa*, respectively, is not known.

Soluble Formate Dehydrogenase (S-FDH)

Formate is an alternative one-carbon source for autotrophic growth of *R. eutropha* H16 [Bowien and Schlegel, 1981]. The product of formate oxidation, CO_2 , is assimilated by the CBB pathway [Bowien and Kusian, 2002]. Organoautotrophic growth with formate depends on a soluble Mo-dependent formate dehydrogenase termed S-FDH [Friedebold and Bowien, 1993]. S-FDH activity was also present during growth on oxalate [Friedrich et al., 1979]. The S-FDH couples formate oxidation with direct reduction of NAD^+ , thus providing reducing equivalents for autotrophic CO_2 fixation. If molybdenum is replaced by tungsten in the growth medium, only traces of S-FDH activity can be detected and organoautotrophic growth is strongly impaired. The genes for S-FDH are organized in an operon of five genes *fdsG*, *-B*, *-A*, *-C*, *-D* on chromosome 1 [Oh and Bowien, 1998]. The amino acid sequence of the catalytic subunit FdsA contains signatures for binding of a molybdenum cofactor and four 4Fe-4S clusters. FdsB contains motifs for binding of a 4Fe-4S cluster, FMN, and NAD. FdsG may contain a single 2Fe-2S center. In agreement with these data, 0.71 mol molybdopterin guanine dinucleotide, 0.9 mol FMN, 25 mol iron, and 20 mol acid-labile sulfur were determined for the purified heterotetramer [Friedebold and Bowien, 1993; Friedebold et al., 1995]. Notably, the S-FDH subunits show sequence similarity to the diaphorase module of the SH: FdsB and FdsG are similar to HoxF, while the N-terminus of FdsA is similar to HoxU [Oh and Bowien, 1998]. While FdsD has been established as the fourth subunit of the S-FDH, the biological role of FdsC is unclear. FdsC belongs to the FdhD/NarQ family of proteins that are involved in formation of active FDH enzymes in several bacteria [Barrett and Riggs, 1982; Glaser et al., 1995; Schlindwein et al., 1990]. A deletion of *fdsC* in *R. eutropha* H16 did not alter S-FDH activity. However, the products of *fdhD* and *B1470* (see below) are 26 and 52% identical to FdsC and thus may serve similar functions.

Another soluble FDH may be encoded by *fdwA* and *fdwB* on chromosome 2. The product of *fdwA* exhibits considerable similarity (36% identical residues) over the entire sequence to the FdsA subunit of the NAD-dependent formate dehydrogenase. The closest paralog (32% identical residues) of the *fdwB* product is HoxF, the NAD^+ -reducing subunit of the SH. *B1699*, located adjacent to *fdwB*, encodes a putative Zn-dependent alcohol dehydro-

genase. Thus it is tempting to speculate that the *B1699* product catalyzes an NADH-dependent reduction of a carbonyl substrate to an alcohol, while the *fdwA* and *fdwB* products form a dimeric formate dehydrogenase that recycles NADH at the expense of formate oxidation to CO_2 . The *oxlT* gene, which is located immediately upstream of *fdwB*, encodes a putative oxalate/formate antiporter which may provide the substrate for this FDH.

Membrane-Bound Formate Dehydrogenase (M-FDH)

While S-FDH is formed only in formate-induced cells, M-FDH activity was detectable under various growth conditions [Burgdorf et al., 2001]. Since M-FDH activity was also detected in the presence of tungsten, it was proposed that both a W-containing and a Mo-containing M-FDH are formed. This hypothesis is corroborated by the fact that a distinct W-dependent activity is present under lithoautotrophic and energy-limiting conditions, while the Mo-dependent activity was detected only under organoautotrophic conditions [Bömmers, 1995; Burgdorf, 1998]. In fact, two gene clusters with the coding capacity for membrane-bound FDH enzymes are present in the genome. One of these regions is located on chromosome 1 and includes three genes, *fdhA1*, *fdhB1*, and *fdhC*, which encode a catalytic subunit, an iron-sulfur subunit, and a transmembrane cytochrome *b* subunit, respectively. In addition, an accessory gene *fdhD* is present in this region. The second FDH gene cluster, located on chromosome 2, comprises *fdoG*, *fdoH*, and *fdoI*. The products of these genes show only moderate similarity to the products of *fdhA1* (30% identical), *fdhB1* (24% identical), and *fdhC* (27% identical), respectively. There is no FdhD paralog encoded in this region, however, *fdoI* lies next to a gene *fdhE*. Like FdhD, FdhE is needed for formation of active FDH in *E. coli* [Schlindwein et al., 1990]. In conclusion, the genetic data agree with the presence of two distinct membrane-bound FDHs. On the basis of the primary structure, it is not possible to decide whether these enzymes use Mo or W in their active site. Mo- and W-FDH are rather similar even on the 3D-structural level [Moura et al., 2004]. Furthermore, it should be noted that there are examples of MoCo enzymes that keep their activity even if W replaces Mo at their active site [Moura et al., 2004]. For instance, such a behavior has been reported for FDH from *Desulfovibrio alaskensis* NCIMB 13491 [Bron-dino et al., 2004].

The products of three additional genes (*A3292*, *B1471*, *cbbB*) are distantly related to FDH catalytic subunits. No additional FDH-specific functions are encoded adjacent to *A3292* and *cbbB*. The *cbbB* gene is part of the *cbb* op-

eron on chromosome 1, however, neither a role in CO₂ fixation nor in formate oxidation could be associated with *cbbB* [Bömmer et al., 1996]. The *B1471* gene lies adjacent to genes encoding a paralog of FdsC (*B1470*) and a MoeA paralog (*moeA3*). The latter one may be involved in biosynthesis of a molybdenum cofactor. Interestingly, the products of both *B1471* and *cbbB* are similar to YdeP from *E. coli* and *Shigella flexneri*. YdeP is a putative molybdenum-containing oxidoreductase that is involved in acid resistance in both organisms [Masuda and Church, 2002; Oglesby et al., 2005].

CO Dehydrogenase

The genome contains 14 genes encoding putative members of the molybdenum hydroxylase superfamily which includes, amongst others, xanthine dehydrogenase and the aerotolerant molybdenum-dependent class of carbon monoxide dehydrogenases (Mo-CODH) [Hille, 1996, 2005]. The products of nine of these genes (*coxL1*, *qorL1*, *qorL2*, *coxL3*, *hcrA*, *xdhB*, *xdhB2*, *A0928*) are 39–23% identical to the catalytic subunit CoxL of the CODH from *Oligotropha carboxidovorans*. The gene *coxL1*, encoding the product with the highest similarity, is part of a large cluster that encodes putative flavoprotein (*coxM*) and iron-sulfur protein (*coxS*) subunits as well as orthologs of *O. carboxidovorans* CoxD, -E, -G, and -F. This arrangement suggests that the minimal genetic information for expression of a Mo-CODH is present in *R. eutropha* H16. However, a critical inspection revealed that neither CoxL1 nor one of the other CoxL orthologs in *R. eutropha* H16 contains the conserved cysteine residue that ligates the copper ion in the [CuSMoO₂]-active site of *O. carboxidovorans* CoxL [Dobbek et al., 2002]. Therefore, a physiological role of these gene products in oxidation of CO is questionable. Moreover, growth of *R. eutropha* H16 on CO as the sole carbon and energy source has not yet been demonstrated.

Aerobic Respiration

Oxidative phosphorylation of *R. eutropha* H16, coupled to various substrates, has been investigated by whole-cell proton translocation and growth yield measurements [Bongers, 1967; Probst, 1980]. Cells grown either lithoautotrophically or heterotrophically exhibited similar H⁺/O ratios of approximately 6–7 and 8, respectively [Jones et al., 1975; Probst, 1980]. Thus, three to four protons are translocated across the cytoplasmic membrane for each electron used to form water, indicating the presence of up

to four coupling sites with an H⁺/e⁻ ratio of 1. In combination with data from various inhibitor studies (including HQNO, antimycin A, rotenone, and myxothiazol), it was concluded that the respiratory chain involves NADH dehydrogenase, succinate dehydrogenase, a bc₁ complex and at least three terminal oxidases branching either at the quinol or at the cytochrome *c* level.

NADH Dehydrogenase

NADH dehydrogenase (complex I, NADH:quinone oxidoreductase, NDH-1) is a large membrane-bound protein complex of 13–14 subunits [Friedrich et al., 1998; Yagi et al., 1998] that couples oxidation of NADH with proton translocation. The NDH-1 of *R. eutropha* H16 is encoded on chromosome 1 by 14 genes *nuoA* to *nuoN*. The amino acid sequences of the corresponding gene products show considerable sequence similarity (in a range between 30 and 50% identity) to the respective orthologs from *E. coli* and *P. denitrificans*, thus suggesting an orthodox composition of the enzyme complex. Iron-sulfur clusters N2, N3, and N4 of NDH-1 were tentatively identified by EPR spectroscopy in membrane preparations [Kömen et al., 1991b]. A midpoint potential of $E_{m7} = -130$ mV was determined for the N2 cluster. The NADH dehydrogenase activity, as determined from the membrane fraction, exhibited an unusually high insensitivity towards the inhibitor rotenone [Kömen et al., 1991b]. Thus a peculiar type of NDH-1 was supposed to be present. However, a second NADH dehydrogenase of the NDH-2 type is encoded on chromosome 1 by gene *A2740*. NDH-2 is a single-subunit enzyme that contains FAD, does not conserve the energy of NADH oxidation, and is insensitive to rotenone inhibition [Melo et al., 2004]. Therefore, the expression of *A2740* provides an alternative explanation for the observed rotenone effect. The presence of genes for both NDH-1 and NDH-2 is known for several bacteria (e.g., *E. coli* [Bjorklof et al., 2000]). According to a recent classification [Melo et al., 2004], the enzyme of *R. eutropha* H16 belongs to NDH-2 group A.

Other NADH-Dependent Enzymes Related to Electron Transport

H⁺-transhydrogenase couples the transfer of hydride ion equivalents between NADH and NADPH to proton translocation across a membrane [Jackson, 2003]. The enzyme is a dimer of two identical heterotrimers and is attached to the cytoplasmic membrane by the subunit dII. Under most physiological conditions the enzyme operates in the direction of NADPH formation to provide

reducing equivalents for biosynthetic purposes or glutathione reduction. The reaction is reversible, and NADPH oxidation can be used in some cases to fuel the respiratory chain [Mercer et al., 1999]. Membrane-bound transhydrogenase activity has been detected in heterotrophically grown *R. eutropha* H16 cells which oxidize NADH at a faster rate than NADPH (NADPH/NADH ratio = 0.13) [Jones et al., 1975; Probst, 1980]. The activity was enhanced by addition of exogenous NAD⁺, indicating that transhydrogenase activity is capable to saturate NADH dehydrogenase. Interestingly, membrane-bound transhydrogenase activity was completely absent from cells grown anaerobically on nitrate as electron acceptor [Drozd, 1977]. Four putative transhydrogenase gene regions are apparent from the genome sequence: three of them are found on chromosome 1 (PntA1: A0850, A0851, A0852; PntA2: A1264, A1265, A1266; PntA3: A3128, A3130, A3131) and one is present on chromosome 2 (PntA4: B1714, B1715). The PntA3 region has been identified previously [Hoppensack et al., 1999]; the genes for subunit dI and the membrane anchor dII are separated by a gene *panD* coding for L-aspartate-1-decarboxylase which presumably forms a transcriptional unit with the transhydrogenase genes. The PntA4 region may encode a soluble enzyme since a gene for subunit dII is absent. However, physiological evidence for such an activity is not available. The reason for the presence of redundant transhydrogenase genes is unclear. Possibly the isoenzymes are adapted to distinct metabolic pathways that need high amounts of NADPH like, for example, formation of poly- β -hydroxy-alkanoates [Steinbüchel and Schlegel, 1991]. In fact, overproduction of transhydrogenase has been shown to improve production of poly(3-hydroxybutyrate) in *E. coli* [Sanchez et al., 2006].

The one-electron reduction of quinones leads to semi-quinone intermediates which are known to stress the cell due to the formation of reactive oxygen species [O'Brien, 1991]. In mammals, NAD(P)H quinone oxidoreductase 1 (NQO1) is believed to compete with potentially toxic one-electron reduction pathways by a two-electron reduction of a variety of quinone substrates [Lind et al., 1990]. Bacterial homologs of this enzyme, called MdaB, have been characterized [Adams and Jia, 2005; Wang and Maier, 2004]. In *R. eutropha* H16, putative MdaB homologs are encoded on chromosome 1 (A0616) and chromosome 2 (B2315). However, a cognate quinone monooxygenase gene (*ygiN*) that forms an operon with *mdaB* in *E. coli* appears to be absent. In *E. coli*, MdaB and quinone monooxygenase form a quinone redox cycle [Adams and Jia, 2005]. On the basis of the current data it is question-

able if such a cycle operates in *R. eutropha* H16. Two-electron transfer from NADH or NADPH to quinone substrates is also catalyzed by quinone oxidoreductase (QOR), a member of the superfamily of medium-chain dehydrogenase/reductases [Persson et al., 1994]. Besides QOR, this superfamily includes Zn-dependent alcohol dehydrogenases (Zn-ADH), archaeal glucose dehydrogenase, and others. The genome encodes 15 QOR-like proteins. None of them contains the conserved Zn-binding residues of Zn-ADH, but eight of them (A0632, A2079, A2377, B1004, B1031, B1340, B2004, B2520) contain a tyrosine (Y52 in *E. coli* QOR) that might be important for enzyme activity [Edwards et al., 1996]. Therefore, at least some of the putative QOR proteins may have a function in protecting the cell by quinone reduction. In fact, an NADH-dependent menadione reductase activity has been reported to be present in *R. eutropha* [Repaske and Lizotte, 1965], however, no exact strain designation was given in this study.

Succinate Dehydrogenase

Succinate dehydrogenase (complex II, succinate:quinone reductase, SQR) catalyzes the two-electron oxidation of succinate to fumarate, with quinol being the electron acceptor [Horsefield et al., 2004; Lancaster, 2002]. The reverse reaction is catalyzed by fumarate reductase (QFR). Both activities are reversible, and hence SQR and QFR belong to the same enzyme family (EC 1.3.99.1). However, while QFR is involved in anaerobic respiration with fumarate, SQR is part of the citric acid cycle. The presence of SQR activity in *R. eutropha* H16 was demonstrated with crude extracts of cells grown heterotrophically on various carbon sources (6–31 nmol mg⁻¹ min⁻¹) [Gläser and Schlegel, 1972] and with membrane preparations of lithoautotrophically grown cells (277 nmol mg⁻¹ min⁻¹) [Kömen et al., 1991b]. The SQR is encoded by four genes, *sdhC*, *sdhD*, *sdhA*, *sdhB*, on chromosome 1. The SQR genes are flanked by genes encoding citrate synthase (*cisY*), malate dehydrogenase (*mdh1*) and a citrate lyase subunit (*citE1*). The SQR belongs to the four-subunit type C subgroup [Lemos et al., 2002]. Both transmembrane subunits (SdhC, SdhD) contain the conserved axial ligands for the intermolecular coordination of heme *b*. The iron-sulfur subunit SdhB is 65% identical to *E. coli* SdhB and contains three cysteines and one aspartate for coordination of cluster 1 (2Fe-2S), as well as seven additional cysteine ligands for cluster 2 (4Fe-4S) and cluster 3 (3Fe-4S). The midpoint potentials of these clusters have been tentatively determined by EPR titration of membrane fractions with $E_{m7} = +30$ mV, -280 mV, and $+100$ mV, re-

spectively. These values are well in the range of known midpoint potentials of other SQRs [Lemos et al., 2002]. The flavoprotein subunit SdhA is 56% identical to *E. coli* SdhA and harbors the flavin-binding site.

Gene *B0204* on chromosome 2 may encode a flavoprotein, since the gene product exhibits limited similarity (25% identical residues) to the flavin domain of the unique soluble fumarate reductase Fcc3 from *Shewanella frigidimarina*. Fcc3 contains, in addition to the flavin domain, an N-terminal domain that binds four c-type hemes. This domain is lacking in the *B0204* gene product. *B0204* is flanked by genes for a LysR-type transcriptional regulator (*B0205*) and a hypothetical membrane protein (*B0203*). The *B0203* product contains six transmembrane segments and an N-terminal domain that contains a [4Fe-4S]-binding motif and is partially similar to the membrane-associated subunit of anaerobic glycerol-3-phosphate dehydrogenase (GlpC) of *E. coli*. These features suggest that the products of *B0203* and *B0204* form a membrane-bound electron transfer complex. The physiological function of this hypothetical complex is probably distinct from fumarate reduction, since anaerobic growth with fumarate as terminal electron acceptor has not been reported for *R. eutropha* H16.

Electron Carriers

Both ubiquinone Q₈ and menaquinone (vitamin K₂) are present in lithoautotrophically grown cells in a ratio of 1:0.025 [Probst and Schlegel, 1976]. Menaquinone was found to dominate under oxygen-limiting conditions. Thus, aerobic respiration depends mainly on ubiquinone, while the quinol oxidases of the denitrification pathway most probably use menaquinone as electron donor. However, reconstitution experiments showed that both quinones can drive oxidative phosphorylation with hydrogen as electron donor [Bongers, 1967]. In contrast to several related β -proteobacteria (e.g. from the genus *Alcaligenes*), *R. eutropha* H16 does not encode blue-copper type single-electron mediators like cupredoxin, azurin, or pseudoazurin [De Rienzo et al., 2000; Farver and Pecht, 1991]. More than 60 genes for putative cytochrome *c* proteins are present in the genome. Of these, 10 are not linked to genes for oxidoreductases and encode soluble proteins <30 kDa that may serve as unspecific electron mediators, and another 10 genes encode membrane-bound proteins. Membrane-associated cytochromes of the respiratory chain have been studied by redox titration of whole membranes [Bernhard et al., 1997; Kömen et al., 1991a, b; Probst and Schlegel, 1976]. Heme components with resolved midpoint potentials are assembled in ta-

ble 1. A cytochrome *c* with $E_{m7} = +270$ mV was found to be loosely attached to the membrane and titrates as a single component with E_{m7} around +300 mV in the periplasmic fraction [Kömen et al., 1991a]. According to its midpoint potential, this component may act as electron donor for one or more cytochrome oxidase(s).

Quinol-Cytochrome *c* Oxidoreductase (*bc*₁ Complex)

Respiration of cells growing exponentially under both lithoautotrophic and heterotrophic conditions was inhibited 35 and 55%, respectively, in the presence of 5 μ M of the *bc*₁ complex inhibitor myxothiazol [Kömen et al., 1991b]. In contrast, respiration of cells from dense cultures did respond only weakly to the inhibitor. This indicates that the respiratory chain adapts to a changing environment, and that the ubiquinol-branch predominates when dioxygen becomes limiting. Compared to *P. denitrificans*, the estimated total amount of *bc*₁ complex in *R. eutropha* H16 was found to be low even during exponential growth. The *bc*₁ complex is encoded on chromosome 1 by the genes *qcrA* ('Rieske' iron-sulfur protein), *-B* (cytochrome *b*), and *-C* (cytochrome *c*₁). Like most *c*-type cytochromes, the *qcrC* product contains a CxxCH motif involved in covalent attachment of its heme group. A *c*-type cytochrome was detected by redox titration of membranes [Kömen et al., 1991a] that may represent QcrC according to its high midpoint potential of $E_{m7} = +335$ mV. The cytochrome *b* subunit QcrB most likely coordinates two *b*-type hemes with midpoint potentials at $E_{m7} = +40$ mV and $E_{m7} = +92$ mV, as determined from heterotrophically grown cells [Bernhard et al., 1997]. QcrA shows signatures for both box I and box II [Darrouzet et al., 1999] that contain the cysteine and histidine ligands of the Rieske high-potential 2Fe-2S iron-sulfur cluster. Based on EPR titration of membrane preparations, a midpoint potential of $E_{m7} = +280$ mV was suggested for this cluster [Kömen et al., 1991a].

Terminal Oxidases

The terminal enzymes of electron transfer chains from aerobic bacteria, called terminal oxidases, catalyze the reduction of dioxygen to water. In general, this reaction is coupled to proton translocation through the cytoplasmic membrane [Brzezinski and Ådelroth, 2006; Wikström and Verkhovsky, 2006]. It has been recognized that most bacterial terminal oxidases, despite differences in their electron donors, subunit composition, and heme types, can be grouped together in the heme-copper oxi-

dase superfamily [Garcia-Horsman et al., 1994; van der Oost et al., 1994]. The catalytic subunit I of a typical cytochrome-oxidizing *aa*₃-type heme copper oxidase (e.g. that of the four-subunit *aa*₃ oxidase of *P. denitrificans*) contains a binuclear heme *a*₃-copper center and an additional heme *a*, while subunit II contains Cu_A. In some enzymes, Cu_A is accompanied by another heme, e.g. heme *c* in the *aa*₃ cytochrome oxidase from *Bacillus subtilis* [Saraste et al., 1991]. Both heme and Cu_A are absent from subunit II of quinol-oxidizing heme-copper oxidases. A special member of the heme-copper oxidase family is the *cbb*₃-type cytochrome oxidase [Pitcher and Watmough, 2004]. Its catalytic subunit I is similar to standard enzymes, while a monoheme and a diheme cytochrome *c*, rather than a Cu_A subunit, guide electrons to the catalytic site. The *cbb*₃ oxidase is believed to share a common ancestor with NO reductase and may represent an early event in the evolution of aerobic microorganisms [Saraste and Castresana, 1994; van der Oost et al., 1994, but see also Pereira et al., 2001]. Many bacteria, including *E. coli*, contain yet another type of terminal oxidase, termed cytochrome *bd*-type quinol oxidase. These enzymes do not belong to the heme copper oxidase superfamily and consist of only two subunits [Jünemann, 1997]. Subunit II harbors the heme *b*-heme *d*-active site; another heme *b* is present in subunit I.

Cyanide-inhibition studies with crude membrane extracts of *R. eutropha* H16 cells revealed that at least three different oxidase activities are present in lithoautotrophically grown cells [Kömen et al., 1991b]. These were attributed to two different cytochrome *c* oxidases and one or more quinol oxidase(s). Cytochrome oxidase activity is in line with the detection of at least two membrane-bound *a*-type hemes with midpoint potentials at $E_{m7} = +255$ mV and $E_{m7} = +350$ mV [Kömen et al., 1991a], indicating the presence of a cytochrome *aa*₃ oxidase. Furthermore, a cytochrome *o* was observed by optical spectroscopy that might be derived from a quinol oxidase [Probst and Schlegel, 1976]. Quinol oxidase activity increased during growth under oxygen-limiting conditions. In addition, a heme *d* signal (maximum at 627 nm) was detected in low-temperature (77 K) optical redox difference spectra [Kömen et al., 1991a]. This signal was increased in membranes from cells in the late-logarithmic growth phase. Heme *d* thus seems to be a constituent of a *bd*-type oxidase that is up-regulated during oxygen limited conditions, as known for the *bd* oxidase of *E. coli* [Jünemann, 1997]. Like the *E. coli* enzyme, which is not significantly inhibited by cyanide concentrations up to 0.5 mM [Kita et al., 1984], the quinol-oxidase activity of *R. eutropha*

H16 was not sensitive to cyanide inhibition and exhibited a lower K_m value compared to the cytochrome *c* oxidase activity [Kömen et al., 1991b].

The observation of multiple terminal oxidase activities corresponds with the genomic information for eight distinct terminal oxidases. The *cta*-gene cluster on chromosome 1 encodes most probably an *aa*₃-type oxidase since, within this cluster, genes for heme O synthase (*ctaB*) and heme A synthase (*ctaA*) are present. The genes *ctaC*, *ctaD*, and *ctaE* encode subunits II, I, and III, respectively. An ortholog of the 50-amino-acid fourth subunit detected in the crystal structure of *aa*₃ oxidase from *P. denitrificans* [Iwata et al., 1995], is not encoded in the *cta* gene region. However, two genes, A0344 and A0346, encode proteins of 43 and 74 amino acids, respectively, that contain predicted transmembrane segments and thus may represent additional subunits. Two members of the ScoI/SenC family and one member of the SurF1 family are also encoded in this region. SurF1 is a factor involved in the biogenesis of cytochrome *c* oxidase in mammals [Zhu et al., 1998]. ScoI from *Saccharomyces cerevisiae* is supposed to deliver copper to the Cu_A site of cytochrome oxidase [Rentzsch et al., 1999]. It should be noted, however, that the ScoI homolog SenC in *Rhodobacter capsulatus* affects the assembly of a *cbb*₃-type cytochrome *c* oxidase that lacks Cu_A [Swem et al., 2005] and thus may serve a more general function. A second cytochrome oxidase is encoded by the *cox* genes on chromosome 2. The genes *coxM* and *coxN* code for the subunits II and I, respectively. The products of both *coxO* and *coxP* resemble a subunit III. A putative fourth subunit with three membrane-spanning segments may be encoded by *coxQ*. Nothing is known about the heme composition of this enzyme; it is tentatively assigned as *bb*₃ oxidase. The products of both *ctaC* and *coxM* deviate from standard subunits II of heme copper oxidases by the presence of a C-terminal extension. These extensions harbor a cytochrome *c* signature, indicating that both subunits contain a heme *c* in addition the usual Cu_A site. Three additional heme-copper oxidases, tentatively assigned as *bo*₃-type oxidases, are encoded in the genome by the clusters *cyo1*, *cyo2*, and *cyo3*. The three *cyo* clusters exhibit a similar composition of four genes *cyoA*, *-B*, *-C*, *-D* that encode subunits II, I, III, and a putative fourth subunit. Each of the *cyoA* gene products lacks the conserved ligands of Cu_A present in subunit II of cytochrome oxidases, indicating that these enzymes are quinol oxidases. The clusters *cyo1* and *cyo2* are present on chromosome 1, while the *cyo3* cluster is located on chromosome 2. All enzymes listed above contain the conserved amino acids for the

D- and K-channel in subunit I and thus belong to the type A1 subclass [Pereira et al., 2001].

Four genes *ccoN*, *-O*, *-Q*, *-P* on chromosome 1 encode a *cbb₃*-type cytochrome oxidase. In *Bradyrhizobium japonicum*, this enzyme operates at extremely low partial pressures of oxygen [Preisig et al., 1996]. Interestingly, an ortholog of the anaerobic regulator FNR from *E. coli* is encoded downstream of the *cco*-gene region, and a consensus-binding motif for FNR (TTGAC N4 ATCAA) is located 75 bp upstream of the gene for the catalytic subunit (*ccoN*). Therefore, it is tempting to speculate that *R. eutropha* H16 produces the *cbb₃* oxidase under oxygen-limited conditions. The remaining two oxidase gene regions are located on chromosome 2 and encode alternative *bd*-type oxidases. The presence of these genes is in line with biochemical data (see above), thus supporting the view that one or more *bd*-type oxidases are formed by *R. eutropha* in the late logarithmic growth phase.

Anaerobic Metabolism

Denitrification is an alternative respiration process in which oxidized nitrogen compounds are used as terminal electron acceptors [Zumft, 1997]. Four terminal oxidoreductases, namely nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR), and nitrous oxide reductase (NOS), catalyze the reduction of nitrate, nitrite, nitric oxide, and nitrous oxide, respectively. The obligate formation of nitric oxide distinguishes denitrification from the ammonification pathway, which ultimately leads to ammonia as the product of nitrite reduction [Cole, 1996; Simon, 2002]. Denitrification by *R. eutropha* H16 was described more than 30 years ago [Pfitzner and Schlegel, 1973] and was found to depend on pHG1 [Römermann and Friedrich, 1985]. A pHG1-free mutant grown on nitrate medium accumulated only low amounts of nitrite and showed very poor growth. This phenotype was initially ascribed to the lack of denitrification enzymes. Paradoxically, the genome sequence revealed that chromosome 2 codes for all key enzymes of denitrification except nitrous oxide reductase. Later studies showed that the denitrification defect of a megaplasmid-free mutant was mainly due to the lack of two genes *nrdD* and *nrdG* that code for an anaerobic class III ribonucleotide reductase (RNR) [Siedow et al., 1999]. Interestingly, trans-complementation with *nrdD* and *nrdG* did not only restore denitrification of a mutant devoid of pHG1, but also converted the closely related non-denitrifier *Alcaligenes hydrogenophilus* into a denitrifier. Apparently,

genes for NAR, NIR, and NOR are expressed in *A. hydrogenophilus* but are useless in the absence of an anaerobic RNR. The denitrification defect of a megaplasmid-free mutant of *R. eutropha* H16 could also be relieved by supplementation of the growth medium with cobalamin [A. Pohlmann and B. Friedrich, pers. commun.], indicating that under these conditions a cobalamin-dependent class II RNR became active. This enzyme is encoded by A2390 on chromosome 1.

Nitrate Reductase

Three distinct types of nitrate reductases termed NAS, NAR, and NAP are known from bacteria, all of which depend on the bis-molybdopterin guanine dinucleotide cofactor [Richardson et al., 2001]. The assimilatory NAS resides in the cytoplasm and is involved in NADH-dependent reduction of nitrate. NAR is a membrane-bound quinol-oxidizing enzyme that is involved in anaerobic nitrate respiration. The catalytic subunit of NAR faces the cytoplasm. In contrast, the catalytic site of NAP is located in the periplasm [Potter et al., 2001]. NAP is linked via additional integral membrane subunits to the respiratory chain and uses quinol as the electron donor. The enzyme seems to serve different physiological functions in bacteria, including nitrate respiration and dissipation of excess redox energy.

Activities for all of these enzymes were detected in cells of *R. eutropha* H16 [Warnecke-Eberz and Friedrich, 1993]. In-gel activity-staining revealed molecular weights of 300 and 100 kDa for NAR and NAP, respectively. Biochemical information on NAR is lacking. Two sets of genes, located on pHG1 and on chromosome 2, encode NAR enzymes. In both cases the structural genes *narG*, *-H*, *-J*, *-I* are preceded by two genes for transporters of the major facilitator superfamily (*narK1/narK2* and *narK3/narK4*, respectively) that are supposed to be involved in nitrate and nitrite import and/or secretion [Moir and Wood, 2001]. A regulatory two-component system NarX-NarL, which is presumably involved in nitrate- or nitrite-dependent transcriptional control, is encoded adjacent to *narK1* on pHG1. Genes for NarXL are missing in the immediate vicinity of the *nar* genes on chromosome 2 but are present on this replicon in the neighborhood of the genes for NO reductase. Megaplasmid-free strains are still able to reduce nitrate, and a deletion of the *nar* genes on pHG1 does not impair denitrification [S. Theinert, B. Friedrich and R. Cramm, unpubl. data]. Therefore, both *nar* gene regions appear to be expressed.

The periplasmic NAP is formed independently of the presence of nitrate in the stationary growth phase. A par-

tial biochemical characterization of the enzyme has been reported [Siddiqui et al., 1993]. NAP was purified as a 92-kDa heterodimer of a catalytically subunit NapA and a small subunit NapB. Formate could serve as the electron donor in crude preparations of NAP, indicating that the enzyme assembles with a formate dehydrogenase component in vivo. Interestingly, a 100-kDa protein copurified with NAP but was not further analyzed. A NAP-FDH complex would parallel the formate-to-nitrate respiratory pathway present in *E. coli* [Sawers, 1994]. The genes for NAP (*napE*, -D, -A, B, -C) are located next to a cluster of genes (*ccmA*, -B, -C, -E, -F, -G, -L, -H) for cytochrome *c* maturation [Thöny-Meyer, 2002]. The stop codon of *napC* is spaced from the start codon of *ccmA* by only three bases, suggesting a translational coupling.

Nitrite Reductase

In denitrifying bacteria, the reduction of nitrite to nitric oxide is carried out either by homotrimeric enzymes that contain copper I and copper II (Cu-NIR), or homodimeric enzymes that contain heme *c* and heme *d*₁ (*cd*₁-NIR). Purification of the NIR activity from *R. eutropha* H16 [Sann et al., 1994] yielded a homodimer of 60-kDa subunits. Absorption spectroscopy revealed that the enzyme is a *cd*₁-NIR, showing absorption signals at 420, 522, and 550 nm (heme *c*) and at 464, 616, and 664 nm (heme *d*₁). The genes for nitrite reductase are assembled in a cluster (*nirS*, -C, -F, -D, -G, -H, -J, -N, -E) spanning 8.6 kb on chromosome 2. The first gene of this cluster, *nirS*, is the structural gene encoding *cd*₁-NIR. The remaining genes are presumed to be accessory genes essential for maturation of active nitrite reductase [Zumft, 1997]. In *P. stutzeri* and *P. aeruginosa*, *nirD*, -E, -F, -G, -H, -J have been implicated in formation of heme *d*₁ and/or insertion of heme *d*₁ into the NirS apoenzyme. Nitrite reductase is the only key enzyme of denitrification that is not encoded on pHG1. However, remnants of a gene for a Cu-NIR downstream of the *nor* genes on pHG1 suggest that this replicon has lost the coding capacity for a complete denitrification pathway.

NO Reductase

Several distinct activities for the reduction of NO to nitrous oxide are known to be present in bacteria. Cytoplasmic flavohemoglobins [Poole and Hughes, 2000] and flavorubredoxins [Saraiva et al., 2004; Vicente and Teixeira, 2005] are believed to be involved in detoxification of NO. Flavohemoglobins are hemoglobins that contain an additional flavin-containing diaphorase domain and act as NO-dioxygenase under oxic or NO reductase un-

der anoxic conditions [Gardner and Gardner, 2002]. The presence of a bacterial hemoglobin with diaphorase activity was first reported for *R. eutropha* H16 [Probst and Schlegel, 1976; Probst et al., 1979]. The gene for this protein (*fhp*) was later identified on pHG1 [Cramm et al., 1994]. A strain containing a mutagenized *fhp* indeed showed some deviations in the accumulation of denitrification intermediates but was, however, not substantially impaired in growth [Cramm et al., 1994]. In light of the genome sequence, the blurry phenotype of that mutant may be explained by the presence of a second *fhp*-like gene (*hmp2*) on chromosome 1.

Membrane-bound NO-reducing enzymes termed NOR are instrumental in respiration. The catalytic subunit (NorB) of all NORs that have been characterized to date contains one high-spin heme *b* and a binuclear catalytic center of a low-spin heme *b* and a non-heme iron [Zumft, 2005]. The best investigated NORs are heterodimers that contain, in addition to NorB, a heme *c*-containing subunit NorC which channels electrons from the physiological electron donor cytochrome *c* to NorB [Oubrie et al., 2002]. According to the electron source, this type of NOR has been termed cNOR [Hendriks et al., 2000]. A second type of NOR was first characterized from *R. eutropha* H16 [Cramm et al., 1999]. This single-subunit enzyme was termed qNOR since it receives electrons from quinol. Two sets of genes for qNOR are present in the genome, located on pHG1 and on chromosome 2. Only the plasmid-derived enzyme has been purified, however, physiological data show that both regions are expressed and that either of them is sufficient for denitrification [Cramm et al., 1997]. Both regions consist of three genes that code for NOR (*norB*), a protein of unknown function (*norA*) and a transcriptional regulator (*norR*) that activates transcription of *norA* and *norB* in response to NO [Pohlmann et al., 2000].

Nitrous Oxide Reductase

Nitrous oxide reductase (NOS) catalyzes the reduction of nitrous oxide to the final product of denitrification, molecular nitrogen. The enzyme has been characterized in most detail from *Pseudomonas* and *Paracoccus* species as periplasmic multicopper protein that contains Cu_A and an unusual tetranuclear Cu_Z center [Zumft and Kroneck, 2006]. The NOS of *R. eutropha* H16 has not been investigated on the biochemical level. Genes for NOS are located adjacent to the *nor* genes on pHG1. As found in the genomes of other denitrifiers [Philippot, 2002], the structural gene for NOS, *nosZ*, is accompanied by accessory *nos* genes (*nosR*, -D, -F, -Y, -L, -C, -X) that

are thought to be involved in cofactor transport, *nos* gene expression, maturation, and sustainment of enzyme activity [Zumft, 2005]. Except of NosC, the gene products are similar to the respective Nos proteins of *P. denitrificans* in a range of 28–48% identity. NosC is a *c*-type cytochrome that may represent the physiological electron donor of NosZ.

Fermentation Enzymes

Although *R. eutropha* is a strictly respiratory species, several strains including strain H16 and the closely related strain N9A form different NAD-linked dehydrogenases during cultivation under restricted oxygen supply [Steinbüchel et al., 1983]. These enzymes may provide a safety valve to prevent overreduction of the respiratory chain in the absence of terminal electron acceptors. In fact, a broad collection of partially oxidized compounds have been found to be excreted by oxygen-limited cultures of *R. eutropha* strain N9A. Most of these compounds, including ethanol, succinate, formate, acetate, and 2-oxoglutarate, are each formed in a distinct range of respiration rates [Vollbrecht et al., 1979]. The genes for a multifunctional alcohol dehydrogenase (*adh*) and a lactate dehydrogenase (*A0666*) of *R. eutropha* H16 have been cloned and characterized [Jendrossek et al., 1990, 1993]. The promoter region of both genes was found to be similar, however, no data are available about the regulatory mechanism that leads to derepression of *adh* and *ldh* under oxygen deprivation. The genome contains 21 genes that encode putative alcohol dehydrogenases. Of these, 7 (including *adh*) belong to the zinc-containing ADHs, 3 belong to the iron containing ADHs, and 9 belong to the short-chain dehydrogenase superfamily. Gene *A1591* encodes a putative membrane-bound ADH of the cytochrome *c* family.

Concluding Remarks

The energy metabolism of *R. eutropha* H16 is well adapted to life in habitats subject to transient anoxia and fluctuating sources of carbon. Accordingly, the respiratory chain offers flexibility with respect to the source, the allocation, and the ultimate fate of electrons. Analysis of the genome uncovered genes for well-known physiological functions, allowed predictions of previously unidentified components, and suggested the existence of many isoenzymes involved in energy metabolism. Certain iso-

enzymes may be the result of gene duplication events, as exemplified by the nitric oxide reductase-encoding *nor* genes. Many others, however, appear to have different kinetic properties (e.g., terminal oxidases) or metal cofactor content (e.g., formate dehydrogenases, ribonucleotide reductases) thus allowing the organism to cope with rapidly changing dioxygen concentrations and environments with varying metal supply. Oxidation of hydrogen and formate either fuels the respiratory chain or, if mediated by SH and S-FDH, provides a direct source of NADH which is needed for carbon dioxide fixation. The redox state of the cell is balanced by several transhydrogenases, and a number of potential redox valves are present. Redox energy may be dissipated for instance by incomplete fermentation pathways, by the periplasmic nitrate reductase, or by the hydrogenases if they reversely operate in the direction of hydrogen evolution. Some gene products that are discussed in this review show considerable similarity to certain components of energy metabolism but lack functional residues (e.g., the CO dehydrogenase-like CoxL, and the hydrogenase-like HycE). Others like the genes for sulfur oxidation (*sox*) appear to code for intact products but cannot be associated to physiological activities. Future studies must be awaited to clarify if these functions add to the metabolic capacity of *R. eutropha* H16.

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References

- Adams MA, Jia Z: Structural and biochemical evidence for an enzymatic quinone redox cycle in *Escherichia coli*: identification of a novel quinol monooxygenase. *J Biol Chem* 2005; 280:8358–8363.
- Aragno M, Schlegel HG: The mesophilic hydrogen-oxidizing (knallgas) bacteria; in Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds): *The Prokaryotes*. New York, Springer, 1992, pp 344–384
- Bardischewsky F, Fischer J, Holler B, Friedrich CG: SoxV transfers electrons to the periplasm of *Paracoccus pantotrophus* – an essential reaction for chemotrophic sulfur oxidation. *Microbiology* 2006;152:465–472.

- Barrett EL, Riggs DL: *Salmonella typhimurium* mutants defective in the formate dehydrogenase linked to nitrate reductase. *J Bacteriol* 1982;149:554–560.
- Bernhard M, Schwartz E, Rietdorf J, Friedrich B: The *Alcaligenes eutrophus* membrane-bound hydrogenase gene locus encodes functions involved in maturation and electron transport coupling. *J Bacteriol* 1996;178:4522–4529.
- Bernhard M, Benelli B, Hochkoeppler A, Zannoni D, Friedrich B: Functional and structural role of the cytochrome *b* subunit of the membrane-bound hydrogenase complex of *Alcaligenes eutrophus* H16. *Eur J Biochem* 1997;248:179–186.
- Bjorklof K, Zickermann V, Finel M: Purification of the 45-kDa, membrane-bound NADH dehydrogenase of *Escherichia coli* (NDH-2) and analysis of its interaction with ubiquinone analogues. *FEBS Lett* 2000;467:105–110.
- Blakesch M, Paschos A, Theodoratou E, Bauer A, Hube M, Huth S, Bock A: Metal insertion into NiFe-hydrogenases. *Biochem Soc Trans* 2002;30:674–680.
- Bömmers D: Genetic investigation of formate oxidation in *Alcaligenes eutrophus*: characterization of the *cbbB_c* gene (in German); PhD Thesis, Institute for Microbiology and Genetics, Department of Molecular Physiology, University of Göttingen, Göttingen 1995.
- Bömmers D, Schäferjohann J, Bowien B: Identification of *cbbB_c* as an additional distal gene of the chromosomal *cbb* CO₂ fixation operon from *Ralstonia eutropha*. *Arch Microbiol* 1996;166:245–251.
- Bongers L: Phosphorylation in hydrogen bacteria. *J Bacteriol* 1967;93:1615–1623.
- Bowien B, Kusian B: Genetics and control of CO₂ assimilation in the chemoautotroph *Ralstonia eutropha*. *Arch Microbiol* 2002;178:85–93.
- Bowien B, Schlegel HG: Physiology and biochemistry of aerobic hydrogen-oxidizing bacteria. *Annu Rev Microbiol* 1981;35:405–452.
- Brondino CD, Passeggi MC, Caldeira J, Almendra MJ, Feio MJ, Moura JJ, Moura I: Incorporation of either molybdenum or tungsten into formate dehydrogenase from *Desulfovibrio alaskensis* NCIMB 13491; EPR assignment of the proximal iron-sulfur cluster to the pterin cofactor in formate dehydrogenases from sulfate-reducing bacteria. *J Biol Inorg Chem* 2004;9:145–151.
- Brzezinski P, Adeltroth P: Design principles of proton-pumping haem-copper oxidases. *Curr Opin Struct Biol* 2006;16:465–472.
- Burgdorf T: Genetic analysis of formate oxidation and molybdopterin biosynthesis in *Ralstonia eutropha* (in German); PhD Thesis, Department of Molecular Physiology, Georg-August-Universität, Göttingen 1998.
- Burgdorf T, Bömmers D, Bowien B: Involvement of an unusual *mol* operon in molybdopterin cofactor biosynthesis in *Ralstonia eutropha*. *J Mol Microbiol Biotechnol* 2001;3:619–629.
- Burgdorf T, Lenz O, Bührke T, van der Linden E, Jones AK, Albracht SP, Friedrich B: [NiFe]-hydrogenases of *Ralstonia eutropha* H16: modular enzymes for oxygen-tolerant biological hydrogen oxidation. *J Mol Microbiol Biotechnol* 2005;10:181–196.
- Burgdorf T, van der Linden E, Bernhard M, Yin QY, Back JW, Hartog AF, Muijsers AO, de Koster CG, Albracht SP, Friedrich B: The soluble NAD⁺-reducing [NiFe]-hydrogenase from *Ralstonia eutropha* H16 consists of six subunits and can be specifically activated by NADPH. *J Bacteriol* 2005;187:3122–3132.
- Cole J: Nitrate reduction to ammonia by enteric bacteria: redundancy, or a strategy for survival during oxygen starvation? *FEMS Microbiol Lett* 1996;136:1–11.
- Cramm R, Pohlmann A, Friedrich B: Purification and characterization of the single-component nitric oxide reductase from *Ralstonia eutropha* H16. *FEBS Lett* 1999;460:6–10.
- Cramm R, Siddiqui RA, Friedrich B: Primary sequence and evidence for a physiological function of the flavohemoprotein of *Alcaligenes eutrophus*. *J Biol Chem* 1994;269:7349–7354.
- Cramm R, Siddiqui RA, Friedrich B: Two iso-functional nitric oxide reductases in *Alcaligenes eutrophus* H16. *J Bacteriol* 1997;179:6769–6777.
- Darrouzet E, Valkova-Valchanova M, Ohnishi T, Daldal F: Structure and function of the bacterial bc1 complex: domain movement, subunit interactions, and emerging rationale engineering attempts. *J Bioenerg Biomembr* 1999;31:275–288.
- De Rienzo F, Gabdoulline RR, Menziani MC, Wade RC: Blue copper proteins: a comparative analysis of their molecular interaction properties. *Protein Sci* 2000;9:1439–1454.
- Dobbek H, Gremer L, Kiefersauer R, Huber R, Meyer O: Catalysis at a dinuclear [CuSMo(=O)OH] cluster in a CO dehydrogenase resolved at 1.1-Å resolution. *Proc Natl Acad Sci USA* 2002;99:15971–15976.
- Drozd JW: Growth energetics in *Alcaligenes eutrophus* H16. *Proc Soc Gen Microbiol* 1977;4:72.
- Eberz G, Hogrefe C, Kortlüke C, Kamiński A, Friedrich B: Molecular cloning of structural and regulatory hydrogenase (*hox*) genes of *Alcaligenes eutrophus* H16. *J Bacteriol* 1986;168:636–641.
- Edwards KJ, Barton JD, Rossjohn J, Thorn JM, Taylor GL, Ollis DL: Structural and sequence comparisons of quinone oxidoreductase, ζ -crystallin, and glucose and alcohol dehydrogenases. *Arch Biochem Biophys* 1996;328:173–183.
- Farver O, Pecht I: Electron transfer in proteins: in search of preferential pathways. *FASEB J* 1991;5:2554–2559.
- Forte E, Urbani A, Saraste M, Sarti P, Brunori M, Giuffrè A: The cytochrome *cbb₃* from *Pseudomonas stutzeri* displays nitric oxide reductase activity. *Eur J Biochem* 2001;268:6486–6491.
- Friedebold J, Bowien B: Physiological and biochemical characterization of the soluble formate dehydrogenase, a molybdoenzyme from *Alcaligenes eutrophus*. *J Bacteriol* 1993;175:4719–4728.
- Friedebold J, Mayer F, Bill E, Trautwein AX, Bowien B: Structural and immunological studies on the soluble formate dehydrogenase from *Alcaligenes eutrophus*. *Biol Chem Hoppe Seyler* 1995;376:561–568.
- Friedrich B, Schwartz E: Molecular biology of hydrogen utilization in aerobic chemolithotrophs. *Annu Rev Microbiol* 1993;47:351–383.
- Friedrich CG, Bardischewsky F, Rother D, Quentmeier A, Fischer J: Prokaryotic sulfur oxidation. *Curr Opin Microbiol* 2005;8:253–259.
- Friedrich CG, Bowien B, Friedrich B: Formate and oxalate metabolism in *Alcaligenes eutrophus*. *J Gen Microbiol* 1979;115:185–192.
- Friedrich CG, Rother D, Bardischewsky F, Quentmeier A, Fischer J: Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? *Appl Environ Microbiol* 2001;67:2873–2882.
- Friedrich T, Abelman A, Brors B, Guenebaut V, Kintscher L, Leonard K, Rasmussen T, Scheide D, Schlitt A, Schulte U, Weiss H: Redox components and structure of the respiratory NADH:ubiquinone oxidoreductase (complex I). *Biochim Biophys Acta* 1998;1365:215–219.
- Garcia-Horsman JA, Barquera B, Rumbley J, Ma J, Gennis RB: The superfamily of heme-copper respiratory oxidases. *J Bacteriol* 1994;176:5587–5600.
- Gardner AM, Gardner PR: Flavohemoglobin detoxifies nitric oxide in aerobic, but not anaerobic, *Escherichia coli*. Evidence for a novel inducible anaerobic nitric oxide-scavenging activity. *J Biol Chem* 2002;277:8166–8171.
- Gläser H, Schlegel HG: Synthesis of enzymes of the tricarboxylic acid cycle in *Hydrogenomonas eutropha* strain H16 (in German). *Arch Microbiol* 1972;86:315–325.
- Glaser P, Danchin A, Kunst F, Zuber P, Nakano MM: Identification and isolation of a gene required for nitrate assimilation and anaerobic growth of *Bacillus subtilis*. *J Bacteriol* 1995;177:1112–1115.
- Gottschalk G, Eberhardt U, Schlegel HG: Utilization of fructose by *Hydrogenomonas* H16 (I). *Arch Microbiol* 1964;48:95–108.
- Hendriks J, Oubrie A, Castresana J, Urbani A, Gemeinhardt S, Saraste M: Nitric oxide reductases in bacteria. *Biochim Biophys Acta* 2000;1459:266–273.

- Hille R: The mononuclear molybdenum enzymes. *Chem Rev* 1996;96:2757–2816.
- Hille R: Molybdenum-containing hydroxylases. *Arch Biochem Biophys* 2005;433:107–116.
- Hoppensack A, Rehm BH, Steinbüchel A: Analysis of 4-phosphopantetheinylation of polyhydroxybutyrate synthase from *Ralstonia eutropha*: generation of β -alanine auxotrophic Tn5 mutants and cloning of the *panD* gene region. *J Bacteriol* 1999;181:1429–1435.
- Horsefield R, Iwata S, Byrne B: Complex II from a structural perspective. *Curr Protein Pept Sci* 2004;5:107–118.
- Iwata S, Ostermeier C, Ludwig B, Michel H: Structure at 2.8 Å resolution of cytochrome *c* oxidase from *Paracoccus denitrificans*. *Nature* 1995;376:660–669.
- Jackson JB: Proton translocation by transhydrogenase. *FEBS Lett* 2003;545:18–24.
- Jendrossek D, Kratzin HD, Steinbüchel A: The *Alcaligenes eutrophus* *ldh* structural gene encodes a novel type of lactate dehydrogenase. *FEMS Microbiol Lett* 1993;112:229–235.
- Jendrossek D, Krüger N, Steinbüchel A: Characterization of alcohol dehydrogenase genes of derepressible wild-type *Alcaligenes eutrophus* H16 and constitutive mutants. *J Bacteriol* 1990;172:4844–4851.
- Johnson BF, Stanier RY: Dissimilation of aromatic compounds by *Alcaligenes eutrophus*. *J Bacteriol* 1971;107:468–475.
- Jones CW, Brice JM, Downs AJ, Drozd JW: Bacterial respiration-linked proton translocation and its relationship to respiratory-chain composition. *Eur J Biochem* 1975;52:265–271.
- Jünemann S: Cytochrome *bd* terminal oxidase. *Biochim Biophys Acta* 1997;1321:107–127.
- Kerstens K, De Ley J: Genus *Alcaligenes* Castellani and Chalmers 1919; in Krieg NR, Holt JG (eds): *Bergey's Manual of Systematic Bacteriology*. Baltimore, Williams & Wilkins, 1984, vol 1, pp 361–373.
- Kita K, Konishi K, Anraku Y: Terminal oxidases of *Escherichia coli* aerobic respiratory chain. II. Purification and properties of cytochrome *b*_{558-d} complex from cells grown with limited oxygen and evidence of branched electron-carrying systems. *J Biol Chem* 1984;259:3375–3381.
- Kleihues L, Lenz O, Bernhard M, Buhke T, Friedrich B: The H₂ sensor of *Ralstonia eutropha* is a member of the subclass of regulatory [NiFe] hydrogenases. *J Bacteriol* 2000;182:2716–2724.
- Knüttel K, Schneider K, Erkens A, Plass W, Müller A, Bill E, Trautwein AX: Redox properties of the metal centres in the membrane-bound hydrogenase from *Alcaligenes eutrophus* CH34. *Bull Pol Acad Sci Chem* 1994;42:495–511.
- Kömen R, Zannoni D, Ingledew WJ, Schmidt K: The electron transport system of *Alcaligenes eutrophus* H16. I. Spectroscopic and thermodynamic properties. *Arch Microbiol* 1991a;155:382–390.
- Kömen R, Zannoni D, Schmidt K: The electron transport system of *Alcaligenes eutrophus* H16. II. Respiratory activities and effect of specific inhibitors. *Arch Microbiol* 1991b;155:436–443.
- Kuhn M, Steinbüchel A, Schlegel HG: Hydrogen evolution by strictly aerobic hydrogen bacteria under anaerobic conditions. *J Bacteriol* 1984;159:633–639.
- Kusian B, Bowien B: Organization and regulation of *cbb* CO₂ assimilation genes in autotrophic bacteria. *FEMS Microbiol Rev* 1997;21:135–155.
- Kusian B, Sultemeyer D, Bowien B: Carbonic anhydrase is essential for growth of *Ralstonia eutropha* at ambient CO₂ concentrations. *J Bacteriol* 2002;184:5018–5026.
- Lancaster CR: Succinate:quinone oxidoreductases: an overview. *Biochim Biophys Acta* 2002;1553:1–6.
- Lemos RS, Fernandes AS, Pereira MM, Gomes CM, Teixeira M: Quinol:fumarate oxidoreductases and succinate:quinone oxidoreductases: phylogenetic relationships, metal centres and membrane attachment. *Biochim Biophys Acta* 2002;1553:158–170.
- Lenz O, Bernhard M, Buhke T, Schwartz E, Friedrich B: The hydrogen-sensing apparatus in *Ralstonia eutropha*. *J Mol Microbiol Biotechnol* 2002;4:255–262.
- Lind C, Cadenas E, Hochstein P, Ernster L: DT-diaphorase: purification, properties, and function. *Methods Enzymol* 1990;186:287–301.
- Masuda N, Church GM: *Escherichia coli* gene expression responsive to levels of the response regulator EvgA. *J Bacteriol* 2002;184:6225–6234.
- Melo AM, Bandejas TM, Teixeira M: New insights into type II NAD(P)H:quinone oxidoreductases. *Microbiol Mol Biol Rev* 2004;68:603–616.
- Mercer NA, McKelvey JR, Fioravanti CF: *Hymenolepis diminuta*: catalysis of transmembrane proton translocation by mitochondrial NADPH→NAD transhydrogenase. *Exp Parasitol* 1999;91:52–58.
- Moir JW, Wood NJ: Nitrate and nitrite transport in bacteria. *Cell Mol Life Sci* 2001;58:215–224.
- Moura JJ, Brondino CD, Trincao J, Romao MJ: Mo and W bis-MGD enzymes: nitrate reductases and formate dehydrogenases. *J Biol Inorg Chem* 2004;9:791–799.
- Nomenclature Committee of the International Union of Biochemistry (NC-IUB): Nomenclature of electron-transfer proteins. Recommendations 1989. *J Biol Chem* 1992;267:665–677.
- O'Brien PJ: Molecular mechanisms of quinone cytotoxicity. *Chem Biol Interact* 1991;80:1–41.
- Oglesby AG, Murphy ER, Iyer VR, Payne SM: Fur regulates acid resistance in *Shigella flexneri* via RyhB and *ydeP*. *Mol Microbiol* 2005;58:1354–1367.
- Oh JI, Bowien B: Structural analysis of the *fds* operon encoding the NAD⁺-linked formate dehydrogenase of *Ralstonia eutropha*. *J Biol Chem* 1998;273:26349–26360.
- Orawski G, Bardischewsky F, Quentmeier A, Rother D, Friedrich CG: The periplasmic thioredoxin SoxS plays a key role in activation in vivo of chemotrophic sulfur oxidation of *Paracoccus pantotrophus*. *Microbiology* 2007;153:1081–1086.
- Oubrie A, Gemeinhardt S, Field S, Marritt S, Thomson AJ, Saraste M, Richardson DJ: Properties of a soluble domain of subunit C of a bacterial nitric oxide reductase. *Biochemistry* 2002;41:10858–10865.
- Pereira MM, Santana M, Teixeira M: A novel scenario for the evolution of haem-copper oxygen reductases. *Biochim Biophys Acta* 2001;1505:185–208.
- Persson B, Zigler JS Jr, Jornvall H: A super-family of medium-chain dehydrogenases/reductases (MDR). Sub-lines including ζ -crystallin, alcohol and polyol dehydrogenases, quinone oxidoreductase enoyl reductases, VAT-1 and other proteins. *Eur J Biochem* 1994;226:15–22.
- Pfützner J, Schlegel HG: Denitrification in *Hydrogenomonas eutropha* strain H 16. *Arch Microbiol* 1973;90:199–211.
- Philippot L: Denitrifying genes in bacterial and Archaeal genomes. *Biochim Biophys Acta* 2002;1577:355–376.
- Pitcher RS, Watmough NJ: The bacterial cytochrome *cbb*₃ oxidases. *Biochim Biophys Acta* 2004;1655:388–399.
- Pohlmann A, Cramm R, Schmelz K, Friedrich B: A novel NO-responding regulator controls the reduction of nitric oxide in *Ralstonia eutropha*. *Mol Microbiol* 2000;38:626–638.
- Pohlmann A, Fricke WF, Reinecke F, Kusian B, Liesegang H, Cramm R, Eitinger T, Ewering C, Potter M, Schwartz E, Strittmatter A, Voss I, Gottschalk G, Steinbüchel A, Friedrich B, Bowien B: Genome sequence of the bioplastic-producing 'Knallgas' bacterium *Ralstonia eutropha* H16. *Nat Biotechnol* 2006;24:1257–1262.
- Poole RK, Hughes MN: New functions for the ancient globin family: bacterial responses to nitric oxide and nitrosative stress. *Mol Microbiol* 2000;36:775–783.
- Potter L, Angove H, Richardson D, Cole J: Nitrate reduction in the periplasm of Gram-negative bacteria. *Adv Microb Physiol* 2001;45:51–112.
- Preisig O, Zufferey R, Thöny-Meyer L, Appleby CA, Hennecke H: A high-affinity *cbb*₃-type cytochrome oxidase terminates the symbiosis-specific respiratory chain of *Bradyrhizobium japonicum*. *J Bacteriol* 1996;178:1532–1538.
- Probst I: *Respiration in Hydrogen Bacteria*. Boca Raton, CRC Press, 1980, vol 2, pp 159–181.
- Probst I, Schlegel HG: Respiratory components and oxidase activities in *Alcaligenes eutrophus*. *Biochim Biophys Acta* 1976;440:412–428.

- Probst I, Wolf G, Schlegel HG: An oxygen-binding flavohemoprotein from *Alcaligenes eutrophus*. *Biochim Biophys Acta* 1979;576:471–478.
- Rentzsch A, Krumbeck-Weiss G, Hofer A, Bartuschka A, Ostermann K, Rödel G: Mitochondrial copper metabolism in yeast: mutational analysis of ScoP involved in the biogenesis of cytochrome *c* oxidase. *Curr Genet* 1999;35:103–108.
- Repaske R, Lizotte CL: The electron transport system of *Hydrogenomonas eutropha*. II. Reduced nicotinamide adenine dinucleotide-menadione reductase. *J Biol Chem* 1965;240:4774–4779.
- Richardson DJ, Berks BC, Russell DA, Spiro S, Taylor CJ: Functional, biochemical and genetic diversity of prokaryotic nitrate reductases. *Cell Mol Life Sci* 2001;58:165–178.
- Römermann D, Friedrich B: Denitrification by *Alcaligenes eutrophus* is plasmid dependent. *J Bacteriol* 1985;162:852–854.
- Sanchez AM, Andrews J, Hussein I, Bennett GN, San KY: Effect of overexpression of a soluble pyridine nucleotide transhydrogenase (UdhA) on the production of poly(3-hydroxybutyrate) in *Escherichia coli*. *Biotechnol Prog* 2006;22:420–425.
- Sann R, Kostka S, Friedrich B: A cytochrome *cd*₁-type nitrite reductase mediates the first step of denitrification in *Alcaligenes eutrophus*. *Arch Microbiol* 1994;161:453–459.
- Saraiva LM, Vicente JB, Teixeira M: The role of the flavodiiron proteins in microbial nitric oxide detoxification. *Adv Microb Physiol* 2004;49:77–129.
- Saraste M, Castresana J: Cytochrome oxidase evolved by tinkering with denitrification enzymes. *FEBS Lett* 1994;341:1–4.
- Saraste M, Metso T, Nakari T, Jalli T, Lauraeus M, Van der Oost J: The *Bacillus subtilis* cytochrome-*c* oxidase. Variations on a conserved protein theme. *Eur J Biochem* 1991;195:517–525.
- Sawers G: The hydrogenases and formate dehydrogenases of *Escherichia coli*. *Antonie Van Leeuwenhoek* 1994;66:57–88.
- Schindwein C, Giordano G, Santini CL, Mandrand MA: Identification and expression of the *Escherichia coli fdhD* and *fdhE* genes, which are involved in the formation of respiratory formate dehydrogenase. *J Bacteriol* 1990;172:6112–6121.
- Schneider K, Cammack R, Schlegel HG, Hall DO: The iron-sulphur centres of soluble hydrogenase from *Alcaligenes eutrophus*. *Biochim Biophys Acta* 1979;578:445–461.
- Schwartz E, Henne A, Cramm R, Eitingen T, Friedrich B, Gottschalk G: Complete nucleotide sequence of pHG1: a *Ralstonia eutropha* H16 megaplasmid encoding key enzymes of H₂-based lithoautotrophy and anaerobiosis. *J Mol Biol* 2003;332:369–383.
- Siddiqui RA, Warnecke-Eberz U, Hengsberger A, Schneider B, Kostka S, Friedrich B: Structure and function of a periplasmic nitrate reductase in *Alcaligenes eutrophus* H16. *J Bacteriol* 1993;175:5867–5876.
- Siedow A, Cramm R, Siddiqui RA, Friedrich B: A megaplasmid-borne anaerobic ribonucleotide reductase in *Alcaligenes eutrophus* H16. *J Bacteriol* 1999;181:4919–4928.
- Simon J: Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiol Rev* 2002;26:285–309.
- Steinbüchel A, Kuhn M, Niedrig M, Schlegel HG: Fermentation enzymes in strictly aerobic bacteria: comparative studies on strains of the genus *Alcaligenes* and on *Nocardia opaca* and *Xanthobacter autotrophicus*. *J Gen Microbiol* 1983;129:2825–2835.
- Steinbüchel A, Schlegel HG: Physiology and molecular genetics of poly(β-hydroxy-alkanoic acid) synthesis in *Alcaligenes eutrophus*. *Mol Microbiol* 1991;5:535–542.
- Swem DL, Swem LR, Setterdahl A, Bauer CE: Involvement of SenC in assembly of cytochrome *c* oxidase in *Rhodobacter capsulatus*. *J Bacteriol* 2005;187:8081–8087.
- Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, Wunschiers R, Lindblad P: Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiol Mol Biol Rev* 2002;66:1–20.
- Thöny-Meyer L: Cytochrome *c* maturation: a complex pathway for a simple task? *Biochem Soc Trans* 2002;30:633–638.
- Van der Linden E, Burgdorf T, Bernhard M, Bleijlevens B, Friedrich B, Albracht SP: The soluble [NiFe]-hydrogenase from *Ralstonia eutropha* contains four cyanides in its active site, one of which is responsible for the insensitivity towards oxygen. *J Biol Inorg Chem* 2004;9:616–626.
- Van der Linden E, Faber BW, Bleijlevens B, Burgdorf T, Bernhard M, Friedrich B, Albracht SP: Selective release and function of one of the two FMN groups in the cytoplasmic NAD⁺-reducing [NiFe]-hydrogenase from *Ralstonia eutropha*. *Eur J Biochem* 2004;271:801–808.
- Van der Oost J, de Boer AP, de Gier JW, Zumft WG, Stouthamer AH, van Spanning RJ: The heme-copper oxidase family consists of three distinct types of terminal oxidases and is related to nitric oxide reductase. *FEMS Microbiol Lett* 1994;121:1–9.
- Vicente JB, Teixeira M: Redox and spectroscopic properties of the *Escherichia coli* nitric oxide-detoxifying system involving flavorubredoxin and its NADH-oxidizing redox partner. *J Biol Chem* 2005;280:34599–34608.
- Vignais PM, Colbeau A: Molecular biology of microbial hydrogenases. *Curr Issues Mol Biol* 2004;6:159–188.
- Volbeda A, Charon MH, Piras C, Hatchikian EC, Frey M, Fontecilla-Camps JC: Crystal structure of the nickel-iron hydrogenase from *Desulfovibrio gigas*. *Nature* 1995;373:580–587.
- Vollbrecht D, Schlegel HG, Stoschek G, Janczikowski A: Excretion of metabolites by hydrogen bacteria. IV. Respiration rate-dependent formation of primary metabolites and of poly-3-hydroxybutanoate. *Eur J Appl Microbiol* 1979;7:267–276.
- Wang G, Maier RJ: An NADPH quinone reductase of *Helicobacter pylori* plays an important role in oxidative stress resistance and host colonization. *Infect Immun* 2004;72:1391–1396.
- Warnecke-Eberz U, Friedrich B: Three nitrate reductase activities in *Alcaligenes eutrophus*. *Arch Microbiol* 1993;159:405–409.
- Wikström M, Verkhovsky MI: Towards the mechanism of proton pumping by the haem-copper oxidases. *Biochim Biophys Acta* 2006;1757:1047–1051.
- Yagi T, Yano T, Di Bernardo S, Matsuno-Yagi A: Prokaryotic complex I (NDH-1), an overview. *Biochim Biophys Acta* 1998;1364:125–133.
- Zhu Z, Yao J, Johns T, Fu K, De Bie I, Macmillan C, Cuthbert AP, Newbold RF, Wang J, Chevrete M, Brown GK, Brown RM, Shoubridge EA: SURF1, encoding a factor involved in the biogenesis of cytochrome *c* oxidase, is mutated in Leigh syndrome. *Nat Genet* 1998;20:337–343.
- Zumft WG: Cell biology and molecular basis of denitrification. *Microbiol Mol Biol Rev* 1997;61:533–616.
- Zumft WG: Biogenesis of the bacterial respiratory Cu_A, Cu-S enzyme nitrous oxide reductase. *J Mol Microbiol Biotechnol* 2005;10:154–166.
- Zumft WG: Nitric oxide reductases of prokaryotes with emphasis on the respiratory, heme-copper oxidase type. *J Inorg Biochem* 2005;99:194–215.
- Zumft WG, Kroneck PM: Respiratory transformation of nitrous oxide (N₂O) to dinitrogen by bacteria and archaea. *Adv Microb Physiol* 2006;52:107–227.