Group of Subsystems: Nitrogen oxides metabolism

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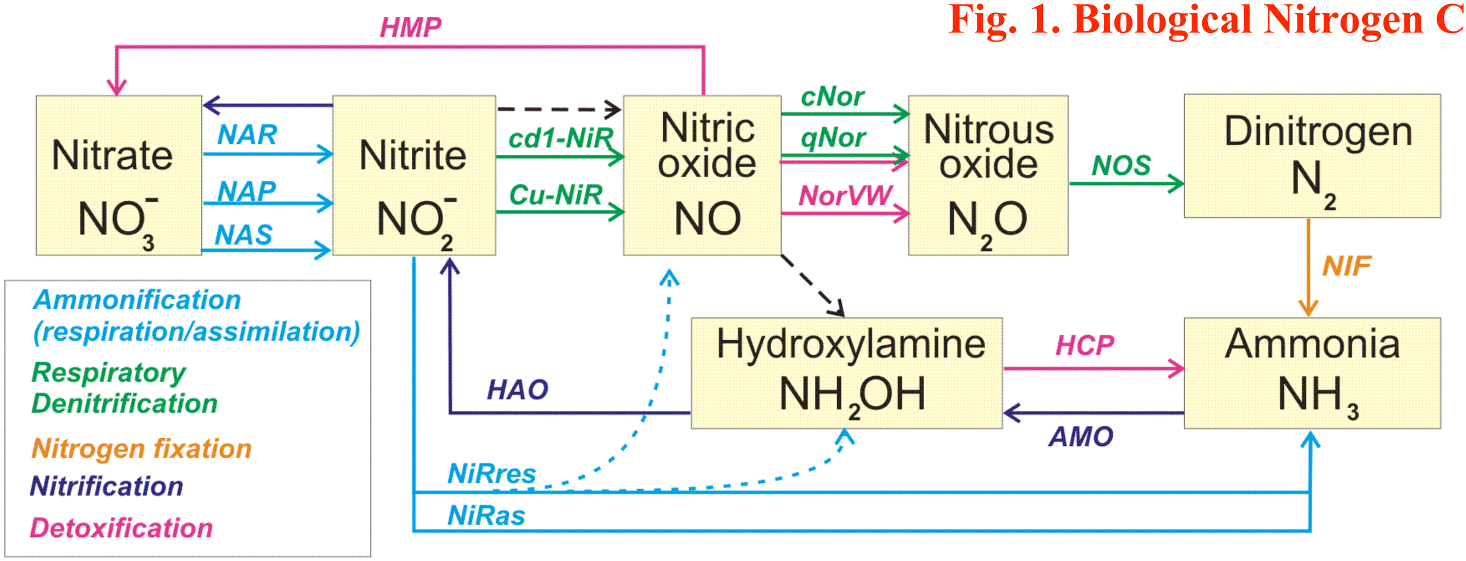
Fig. 1. Biological Nitrogen Cycle



Nitrogen is an essential element in all living organisms. Inter-conversions of nitrogen species between a number of redox states (+5 to -3) form the biogeochemical nitrogen cycle which has multiple environmental impacts and industrial applications [1]. Inorganic nitrogen oxides should be first reduced to ammonium, which can be further incorporated into organic matter via glutamine synthase. Diazotrophic prokaryota possess nitrogenase genes and are able to fix molecular nitrogen from the atmosphere.

On the other hand, bacteria can obtain metabolic energy by redox processes utilizing soluble nitrogen oxides, nitrate and nitrite as terminal respiratory oxidants under oxygen limiting conditions. Two dissimilar pathways of nitrate respiration, ammonification and denitrification, involve formation of a common intermediate, i.e. nitrite, but end in different products, ammonia and gaseous nitrogen oxides (NO or N_2O) or dinitrogen respectively (Fig. 1).

Autotrophic nitrification is a two-step process of an oxidative conversion of ammonia to nitrite via hydroxylamine, carried out by ammonia-oxidizing bacteria, and further oxidation of nitrite to nitrate, performed by nitrite-oxidazing chemolithoautotrophic bacteria.

Finally, the cell should be able to detoxify the exogenously/metabolically produced NO and reactive nitrogen species.

Subsystem: Nitrate and Nitrite ammonification

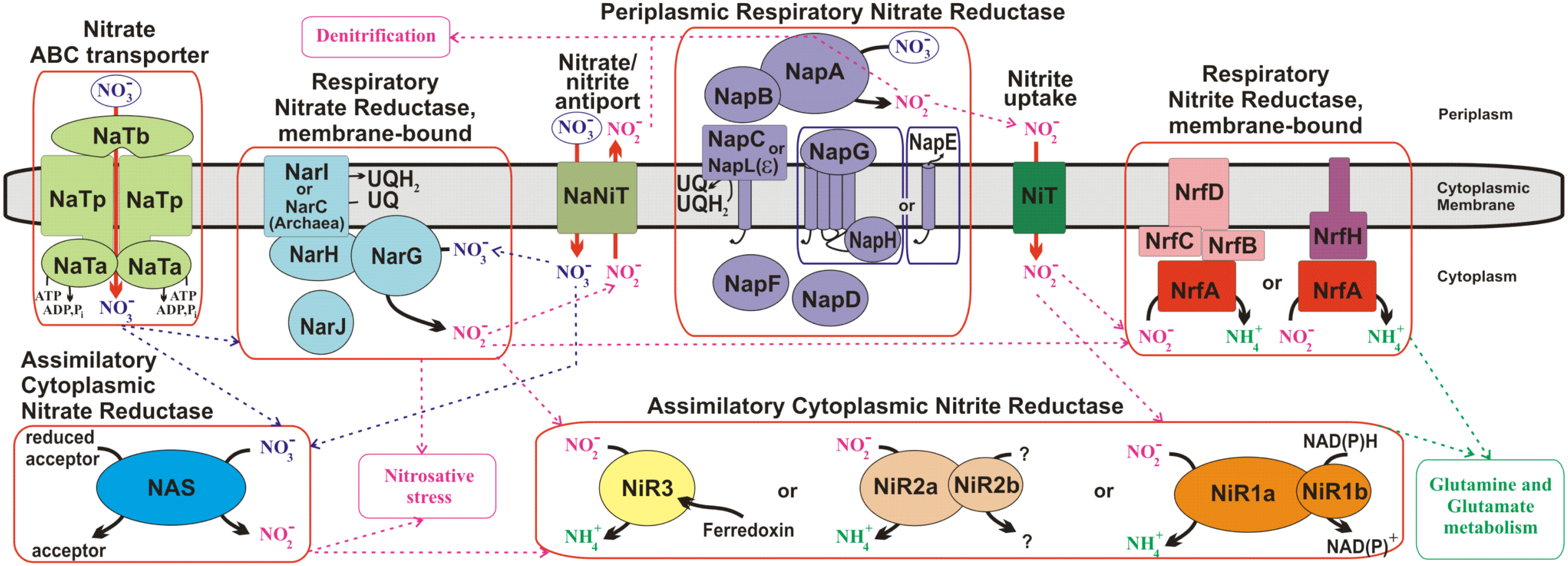
In the first step of this pathway nitrite is formed by one of the three different types of nitrate reductases: soluble assimilatory NAS, membrane-associated respiratory NAR, and periplasmic dissimilatory NAP [2, 3]. NAS is located in the cytoplasmic compartment and participates in nitrogen assimilation (termed NaRas here). NAR is usually a three-subunit complex anchored at the cytoplasmic face of the membrane with its active site located in the cytoplasmic compartment. It is involved in anaerobic nitrate respiration. NAP is a two-subunit complex located in the periplasmic compartment. It is coupled to quinol oxidation via a membrane anchored tetraheme cytochrome.

The members of all three classes of enzymes bind a bis-molybdopterin guanine dinucleotide cofactor at their active sites, but they differ markedly in the number and nature of cofactors used to transfer electrons to this site. Analysis of prokaryotic genomes reveals that different nitrate reductases are phylogenetically widespread.

The next step of ammonification is conversion of nitrite into ammonia by either membrane-bound cytochrome *c* containing respiratory nitrite reductase NrfA, or by one of the three different cytoplasmic assimilatory NiR isoenzymes. In ε- and δ-proteobacteria NrfA forms a stable complex with a transmembrane component NrfH, whereas in γ-proteobacteria NrfH is thought to be replaced by the *nrfBCD* gene products [4]. Among soluble NiRs the siroheme-containing NADPH-dependent enzyme (NirBD in *E.coli*) is the most common one. Cyanobacteria, plants, and some α-proteobacteria possess a distinct ferredoxin-dependent cytoplasmic NiR [5]. Some strictly anaerobic species (e.g. *Clostridia*) have another two-component NiR, which has not yet been characterized [6].

The topological arrangements of nitrate and nitrite reductases in bacteria necessitate synthesis of transporter proteins that carry nitrogen oxyanions across the cytoplasmic membrane. Two types of uptake systems are known to act in assimilation of nitrate (and nitrite): (i) ATP hydrolysis driven ABC transporters, and (ii) secondary transporters reliant on proton motive force, which belong to either nitrite/nitrate transporter family (NarK), nitrite uptake NirC family, or formate/nitrite transporter family [7].

Fig. 2. Nitrate and Nitrite ammonification. Subsystem diagram.



#	Abbrev.	Functional Role	Subset
1	NrfA	Cytochrome c552 precursor (EC 1.7.2.2)	*NiRres
2	NrfB	Cytochrome c-type protein nrfB precursor	
3	NrfC	NrfC protein	
4	NrfD	NrfD protein	
5	NrfH	Cytochrome c nitrite reductase, small subunit NrfH	
6	NiR1a	Nitrite reductase [NAD(P)H] large subunit (EC 1.7.1.4)	*NiRas
7	NiR1b	Nitrite reductase [NAD(P)H] small subunit (EC 1.7.1.4)	
8	NiR2a	Nitrite reductase probable [NAD(P)H] subunit (EC 1.7.1.4)	
9	NiR2b	Nitrite reductase probable electron transfer 4Fe-S subunit (EC	
10	NiR3	Ferredoxin--nitrite reductase (EC 1.7.7.1)	
11	NiTa	Nitrite transporter from formate/nitrite family	*NiT
12	NiTc	Nitrite transporter NirC	
13	NaTb	Nitrate ABC transporter, nitrate-binding protein	*NaT
14	NaTp	Nitrate ABC transporter, permease protein	
15	NaTa	Nitrate ABC transporter, ATP-binding protein	
16	NaNiT	Nitrate/nitrite transporter	NaNiT
17	NAS	Assimilatory nitrate reductase large subunit (EC:1.7.99.4)	NAS
18	NarG	Respiratory nitrate reductase alpha chain (EC 1.7.99.4)	*NAR
19	NarH	Respiratory nitrate reductase beta chain (EC 1.7.99.4)	
20	NarI	Respiratory nitrate reductase gamma chain (EC 1.7.99.4)	
21	NarJ	Respiratory nitrate reductase delta chain (EC 1.7.99.4)	
22	NarC	Respiratory nitrate reductase subunit, conjectural (EC 1.7.99.4)	
23	NapA	Periplasmic nitrate reductase precursor (EC 1.7.99.4)	*NAP
24	NapB	Nitrate reductase cytochrome c550-type subunit	
25	NapC	Cytochrome c-type protein NapC	
26	NapD	Periplasmic nitrate reductase component NapD	
27	NapF	Ferredoxin-type protein NapF (periplasmic nitrate reductase)	
28	NapG	Ferredoxin-type protein NapG (periplasmic nitrate reductase)	
29	NapH	Polyferredoxin NapH (periplasmic nitrate reductase)	
30	NapE	Periplasmic nitrate reductase component NapE	
31	NapL	Periplasmic nitrate reductase component NapL	
32	NaNiRR	Nitrate/nitrite response regulator protein	*Reg
33	NaNiS	Nitrate/nitrite sensor protein (EC 2.7.3.-)	
34	NasT	Response regulator NasT	

Fig. 3. Nitrate and Nitrite ammonification. Subsystem spreadsheet.

Organism	Variant Code	NiR respiratory		NiR assimilatory		NaR assimilatory	NaR respiratory	NaR periplasmic	Nitrite and Nitrate transporters		
		NrfA	NrfBCD or NrfH	NiR1a-6, NiR2a-8, NiR3-10	NiR1b-7, NiR2b-9	NAS	NarGHIJ	NapABCDFGH or NapABCDFE	NiTa-11, NiTc-12	NaTbpa	NaNiT
Escherichia coli K12	1	3980	NrfBCD	3300-6	3301-7		NarGHIJ	NapABCDFGH	3302-12		1455, 1212
Bacillus subtilis	2			331-6, 333-6	330-7	332	NarGHIJ				334, 3739
Vibrio vulnificus CMCP6	3	2794	NrfBCD	3318-6, 3300-6	3317-7, 3299-7	3326		NapABCDFGH, NapCE	3298-11	NaTbpa	
Pseudomonas aeruginosa PAO1	4			1782-6	1781-7	1780	NarGHIJ	NapABCDFE			3876, 1784
Synechocystis sp. PCC 6803	5			2479-10		909				NaTbpa	
Synechococcus sp. WH 8102	5			2468-10		2455					2453, 2454
Xanthomonas campestris	5			1977-6	1978-7	1979					1976
Campylobacter jejuni RM1221	6	1501	NrfH					NapAGHBLD			
Haemophilus influenzae R2846	6	89	NrfBCD					NapABCDFGH			
Yersinia pestis KIM	7			160-6	159-7			NapABCDGH	158-12		
Erwinia carotovora	8	3452	NrfBCD	3058-6, 2949-6	2949-7, 3059-7	2948	NarGHIJ	NapABCDFGH		NaTbpa	3225
Sinorhizobium meliloti 1021	9			5744-6	5745-7	5746		NapABCDFE		NaTbpa	5057
Pirellula sp. 1	10	6159	NrfH	987-6, 215-	2979-7	216, 214				NaTbpa	
Corynebacterium glutamicum	11						NarGHIJ				1143
Thermotoga maritima MSB8	12			389-8	390-9						
Prochlorococcus marinus str. MIT	12			2230-10					2231-11		
Staphylococcus aureus MRSA252	13			2357-6	2356-7		NarGHIJ				2346
Porphyromonas gingivalis W83	14	1560	NrfH								
Symbiobacterium thermophilum	15	698	NrfH			1985, 881					
Vibrio cholerae	16							NapABCDFE			
Chromobacterium violaceum ATCC	17					2229	NarGHIJ				3907, 2545, 2228, 2544
Geobacter metallireducens	18	2519, 2518	NrfH	72-8	71-9		NarGHIJ				2951, 2952
Geobacter sulfurreducens PCA	19	3133, 354	NrfH	1228-8	1229-9						
Shigella dysenteriae M131649	20			2407-6	2408-7		NarGHIJ	NapABCDGH	2409-12		4381

Functional variants: Bacteria can have different combinations of two general types of NiR and three main types of NaR. At least 20 different functional variants of NiR/NaR patterns have been observed in available bacterial genomes. For example:

#1: as in *E. coli*: assimilatory and respiratory NiRs, respiratory membrane-bound and periplasmic NaRs;
#2. as in *B. subtilis*: assimilatory NiR, assimilatory and respiratory NaRs;
#5: as in most cyanobacteria:only assimilatory NaR and NiR.

Another highly variable component of the pathway is the transport systems for nitrate and nitrite ions.

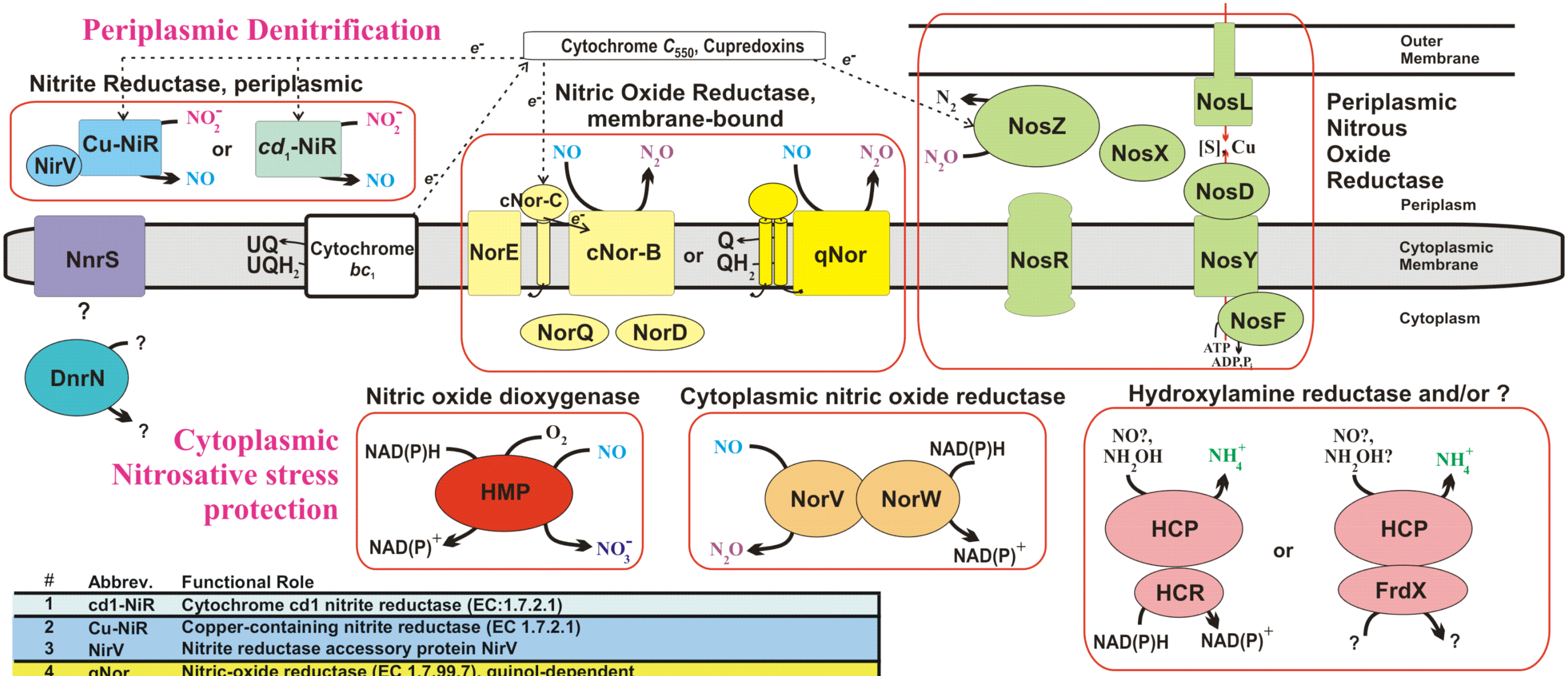
Subsystems: Respiratory Denitrification and and Nitrosative Stress Protection

Denitrification constitutes one of the main branches of the global nitrogen cycle sustained by bacteria [8]. Nitrogen is introduced into the biosphere by fixation of dinitrogen and removed from there again by denitrification. In doing this, denitrification catalyzes successively N-N bond formation in the transformation of its intermediates nitric oxide (NO) and nitrous oxide (N_2O) to the next-lower oxidation state. The bacterial process is nearly exclusively a facultative trait which occurs in periplasm. Its expression is triggered in the cell by the environmental parameters, low oxygen tension, and availability of an N oxide. At the first step, nitrite is formed by one of three different types of nitrate reductases (see Nitrate and Nitrite Ammonification SS), which are shared by the downstream pathways. Next, during denitrification nitrite is reduced to NO by one of the two different types of nitrite reductases (Cu-NiR or cytochrome *cd1*-NiR), then to N_2O by one of the two types of nitric oxide reductase (quinol-dependent qNOR or cytochrome *bc*-type cNOR, [9]), and finally to dinitrogen, using Cu-containing nitrous oxide reductase complex (NOS). Interestingly, the ε-proteobacterium *Wolinella succinogenes* grows anaerobically by respiratory nitrite ammonification but not by denitrification. Nevertheless, it is capable of N_2O reduction to N_2, possesing an active NOS complex [10].

Nitric oxide is a signaling and defense molecule in animals, but bacteria are sensitive to high NO concentrations due to its reactivity and membrane permeability [11]. NO and hydroxylamine (NH_2OH) -- two toxic intermediates in 6-electron reduction of nitrite could be formed during nitrite ammonification [12]. In addition to a classical NO reductase (qNOR or cNOR) occurring in denitrifying species, two other bacterial NO detoxification enzymes have been characterized: an NO reductase (flavorubredoxin NorVW in *Escherichia coli*) and an NO dioxygenase (flavohemoglobin Hmp or Fhp in *E. coli*, *Bacillus subtilis*, *Ralstonia eutropha*, and *Pseudomonas* species) [13]. An unusual redox enzyme, called the hybrid cluster protein (HCP) has been extensively studied both in strictly anaerobic and facultative anaerobic bacteria, where it is mostly induced during conditions of nitrite or nitrate stress. *In vitro* studies demonstrated oxygen-sensitive hydroxylamine reductase activity of the *E. coli* HCP protein, suggesting its possible role in detoxification of reactive by-products of nitrite reduction [14].

Recent comparative analysis of NO protection genes and their transcriptional regulatory signals was used to demonstrate considerable interconnection between various regulons of denitrification and NO detoxification and to identify two new members of the nitrosative stress protection pathway, the hypothetical proteins DnrN and NnrS [13].

Fig. 4. Respiratory Denitrification and Nitrosative Stress Protection. Subsystem diagram.



#	Abbrev.	Functional Role
1	cd1-NiR	Cytochrome cd1 nitrite reductase (EC:1.7.2.1)
2	Cu-NiR	Copper-containing nitrite reductase (EC 1.7.2.1)
3	NirV	Nitrite reductase accessory protein NirV
4	qNor	Nitric-oxide reductase (EC 1.7.99.7), quinol-dependent
5	cNor-B	Nitric-oxide reductase subunit B (EC 1.7.99.7)
6	cNor-C	Nitric-oxide reductase subunit C (EC 1.7.99.7)
7	NorE	Nitric oxide reductase activation protein NorE
8	NorD	Nitric oxide reductase activation protein NorD
9	NorQ	Nitric oxide reductase activation protein NorQ
10	NosZ	Nitrous-oxide reductase (EC 1.7.99.6)
11	NosD	Nitrous oxide reductase maturation protein NosD
12	NosF	Nitrous oxide reductase maturation protein NosF (ATPase)
13	NosY	Nitrous oxide reductase maturation transmembrane protein NosY
14	NosL	Nitrous oxide reductase maturation protein, outer-membrane lipoprotein NosL
15	NosX	Nitrous oxide reductase maturation periplasmic protein NosX
16	NosR	Nitrous oxide reductase maturation protein NosR
17	NnrS	NnrS protein involved in response to NO
18	NnrR	Nitric oxide -responding transcriptional regulator NnrR (Crp/Fnr family)
19	DNR	Nitric oxide -responding transcriptional regulator Dnr (Crp/Fnr family)

#	Abbrev.	Functional Role
1	HMP	Flavohemoprotein
2	HCP	Hydroxylamine reductase (EC 1.7.-.-)
3	HCR	NADH oxidoreductase hcr (EC 1.-.-.-)
4	FrdX	Ferredoxin 3 fused to uncharacterized domain
5	NorV	Anaerobic nitric oxide reductase flavorubredoxin
6	NorW	Nitric oxide reductase FlRd-NAD(+) reductase (EC 1.18.1.-)
7	qNor	Nitric-oxide reductase (EC 1.7.99.7), quinol-dependent
8	DnrN	Nitric oxide-dependent regulator DnrN or NorA
9	NnrS	NnrS protein involved in response to NO
10	NsrR	Nitrite-sensitive transcriptional repressor NsrR
11	NorR	Anaerobic nitric oxide reductase transcription regulator norR
12	HcpR	Hcp transcriptional regulator HcpR (Crp/Fnr family)

Fig. 5. Periplasmic respiratory denitrification. Subsystem spreadsheet.

Organism	Variant Code	cd1-Nitrite reductase	Cu-Nitrite reductase	Quinol NO reductase	Cytochrome bc-type NO reductase (cNoR)					Nitrouse oxide reductase			
		NirS	NirK	qNoR	cNoR-B	cNoR-C	NorE	NorD	NorQ	NosZ	NosD	NosF	NosY
Sinorhizobium meliloti 1021	1		681		694	695	696	692	693	643	644	645	646
Pseudomonas aeruginosa UCBPP-PA	2	5083			5088	5087	5085	5089	5084	976	977	978	979
Neisseria lactamica ST-640	3		563	564						2015	2013	2012	2011
Ralstonia metallidurans	4	3126, 3605		3610						3103	3101	3100	3099
Photobacterium profundum SS9	5				1131	1130	1135	1132	1133	908	909	910	911
Ralstonia eutropha JMP134	6	2868, 2875		2912									
Nitrosomonas europaea ATCC 19718	7		897		1918	1917		1920	1919				
Chromobacterium violaceum	8		2007	3494									
Wolinella succinogenes DSM 1740	9									846, 848	850	855	857
Actinobacillus pleuropneumoniae	10		396										

Functional variants:

#1 and 2: two variants of a complete denitrification pathway from nitrite to N_2, which use different types of nitrite reductase.
#3, 4, 6, 7, 8: five variants of the denitrification pathway from nitrite to N_2O, which use different types of nitrite and NO reductases.
#10: short denitrification pathway from nitrite to NO. #9: solely N_2O reduction pathway. #5: solely NO and N_2O reduction pathway.

Fig. 6. Cytoplasmic nitrosative stress protection. Subsystem spreadsheet.

Organism	Variant Code	NO dioxygenase	NH2OH reductase	Anaerobic NO reductase		NO reductase	N	S
		HMP	HCP	NorV	NorW	qNOR	DnrN	NnrS
Escherichia coli K12	1N	2522	859	2665	2666		4119	
Vibrio vulnificus CMCP6	1S	1196	2634	4202	4203			1625
Yersinia pestis KIM	2N	1295	2771				641	
Vibrio parahaemolyticus	2S	2809	1189					2390
Bacillus subtilis	3	1306						
Staphylococcus aureus NCTC 8325	3N	216					235	
Vibrio cholerae O1	3S	2924						2302
Microbulbifer degradans 2-40	3NS	2172					461	2616
Desulfovibrio desulfuricans G20	4		924					
Clostridium perfringens str. 13	4N		2647				838	
Rhodospirillum rubrum	4S		407					3252
Shewanella oneidensis MR-1	4NS		1266				3904	2551
Chromobacterium violaceum	5S	3488				3494		2711
Ralstonia eutropha JMP134	5NS	5127				2912	2913	2856
Mannheimia succiniciproducens	6N		278			280, 281	1164	
Synechocystis sp. PCC 6803	7					3084		
Neisseria meningitidis Z2491	7NS					1740	1436	1890
Haemophilus influenzae R2866	8N						1056	
Rhodopseudomonas palustris CGA009	8S							1478

Functional variants: Bacteria can have eight different combinations of four known nitrosative stress protection systems (HMP, HCP, qNor and NorVW). These basic variants are further subdivided into sub-variants (marked by additional letters N and S) depending on the presence of the two hypothetical genes *dnrN* and/or *nnrS*, which are often co-regulated or co-localized with nitrosative stress protection and denitrification genes, and thus should play an important functional role in these processes.

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