JOURNAL OF BACTERIOLOGY, Mar. 1995, p. 1409–1413
0021-9193/95/$04.00+0
Copyright © 1995, American Society for Microbiology
Vol. 177, No. 5

The *nasB* Operon and *nasA* Gene Are Required for Nitrate/ Nitrite Assimilation in *Bacillus subtilis*

KOU-ICHIRO OGAWA,[1] EIJI AKAGAWA,[1] KUNIO YAMANE,[1] ZU-WEN SUN,[2] MICHAEL LACELLE,[2] PETER ZUBER,[2] AND MICHIKO M. NAKANO[2]*

Institute of Biological Sciences, University of Tsukuba, Tsukuba-shi, Ibaraki 305, Japan,[1] and Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, Louisiana 71130-3932[2]

Received 1 November 1994/Accepted 31 December 1994

***Bacillus subtilis* can use either nitrate or nitrite as a sole source of nitrogen. The isolation of the *nasABCDEF* genes of *B. subtilis*, which are required for nitrate/nitrite assimilation, is reported. The probable gene products include subunits of nitrate/nitrite reductases and an enzyme involved in the synthesis of siroheme, a cofactor for nitrite reductase.**

The gram-positive, spore-forming bacterium *Bacillus subtilis* can use a number of compounds as sole sources of nitrogen (7), including nitrate, one of the major forms of assimilable nitrogen in the biosphere. Nitrate assimilation involves uptake and reduction of nitrate to nitrite, a reaction catalyzed by nitrate reductase, and further reduction by nitrite reductase to ammonium. Ammonium thus produced in *B. subtilis* is incorporated into carbon skeletons through the activities of glutamine synthetase and glutamate synthase. In *B. subtilis*, very little is known about the biochemistry and genetics of nitrate assimilation. Membrane-bound nitrate reductase activity was detected in *B. subtilis* cells grown in the presence of nitrate with limited aeration (2, 17). Two mutations conferring defects in nitrate assimilation, *narA* (12) and *narB* (29), were localized on the *B. subtilis* genetic map to 320° and 28°, respectively. In this paper, the isolation and analysis of *B. subtilis* DNA from the *narB* locus, containing six genes (now called *nasA*, *nasB*, *nasC*, *nasD*, *nasE*, and *nasF*) (Fig. 1) required for nitrate/nitrite assimilation, are reported.

The *nas* genes were originally identified from an SPβ phage library containing *B. subtilis* chromosomal DNA fused to a promoterless *lacZ* gene (31, 32), as promoter activity which was induced postexponentially by the lipopeptide surfactin (19) in sporulation medium supplemented with glucose (DSG) (20). How surfactin stimulates the expression of this *lacZ* fusion is unknown at present and is currently under investigation. By a procedure previously described (33), the fusion was transferred from SPβ to a plasmid replicon, yielding pZS1. A 0.6-kb fragment possessing the promoter activity was inserted into an integration vector, pMMN13 (chloramphenicol resistant [Cm^r]), yielding plasmids pZS3 and pZS4 (bearing the insert in opposite orientations). A *B. subtilis* strain, JH642, was transformed with pZS4, and the transformant (LAB998) was used for chromosomal mapping of *nas* genes by PBS1 transduction (*B. subtilis* strains and genotypes are listed in Table 1). Transduction linkage to *aroI* and *srfA* (80%) was detected.

To determine the orientation of the observed transcriptional activity, the insert fragments from pZS3 and pZS4 were subcloned in front of the promoterless *lacZ* gene of pTKlac (11). The resulting plasmids, pZS5 and pZS6, were integrated into the SPβ prophage in the *B. subtilis* chromosome (33). The resulting specialized transducing phages were used to transfer the fusion-bearing plasmids into cells of strain JH642. Two strains thus constructed, LAB1166 (SPβ::pZS5) and LAB1167 (SPβ::pZS6), showed a Lac^+ phenotype, suggesting the presence of divergent promoters in the DNA fragment. Both promoter activities were regulated by nitrogen availability, as previously reported (20).

In order to isolate the genes controlled by the promoters, the regions flanking the 0.6-kb DNA fragment were isolated by chromosome walking. The nucleotide sequence of 11.5 kb of DNA was determined with an automated sequencer and DNA of plasmids pZS7, pMMN148, and pMMN159 (Fig. 1). Sequence analysis revealed six open reading frames (ORFs), *nasA*, *-B*, *-C*, *-D*, *-E*, and *-F*, each preceded by a potential ribosome binding site (Fig. 1). ORFs *nasB* to *nasF* are transcribed in the same direction from the P1 promoter, and *nasA* is divergently transcribed from the P2 promoter (20). *nasB* appears to encode a protein of 770 amino acids, the C-terminus-encoding codons of which overlap with the Shine-Dalgarno sequence of *nasC*, encoding a protein of 710 amino acids. *nasD*, which begins approximately 120 bp downstream of the termination codon of *nasC*, is an ORF encoding an 805-amino-acid product. A small ORF, *nasE*, was found 30 bp downstream of *nasD* and putatively encodes a product of 106 amino acids. The product of *nasF*, which starts 65 bp downstream of the *nasE* gene, is a protein of 483 amino acids. *nasF* is immediately followed by a potential ρ-independent transcription termination sequence. Upstream of *nasB* to *-F* and oriented divergently is the transcription unit *nasA*, encoding a 421-residue product. A putative transcription terminator lies 150 bp downstream of *nasA*.

The FASTA algorithm of Lipman and Pearson (15) was used in a computer-aided (DNA star Inc., Madison, Wis.) search for proteins with structures similar to those of the *nas* products. The *nasB* and *nasD* products showed homology to *Escherichia coli* respiratory NADH-nitrite reductase (NirB) (16, 23) and to assimilatory nitrite reductases of *Aspergillus nidulans* (NiiA) (10) and *Klebsiella pneumoniae* (NasB) (13) (Table 2). Both NasB and NasD contain flavin adenine dinucleotide (FAD)-binding and NAD(P)H-binding domains including the conserved GXGXXG motif (27) (amino acids 10 to 15 and 152 to 157 in NasB and NasD). The C-X5-C-Xn-S-X-C-X3-C motif, characteristic of a (4Fe/4S)-siroheme-binding domain (18) in nitrite reductases (6), is found in NasD (amino acids 635 to 679) but is absent in NasB, suggesting that NasD

* Corresponding author. Mailing address: Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, 1501 Kings Highway, Shreveport, LA 71130-3932. Phone: (318) 675-5158. Fax: (318) 675-5180. Electronic mail address: mnakan@nomvs.lsumc.edu.

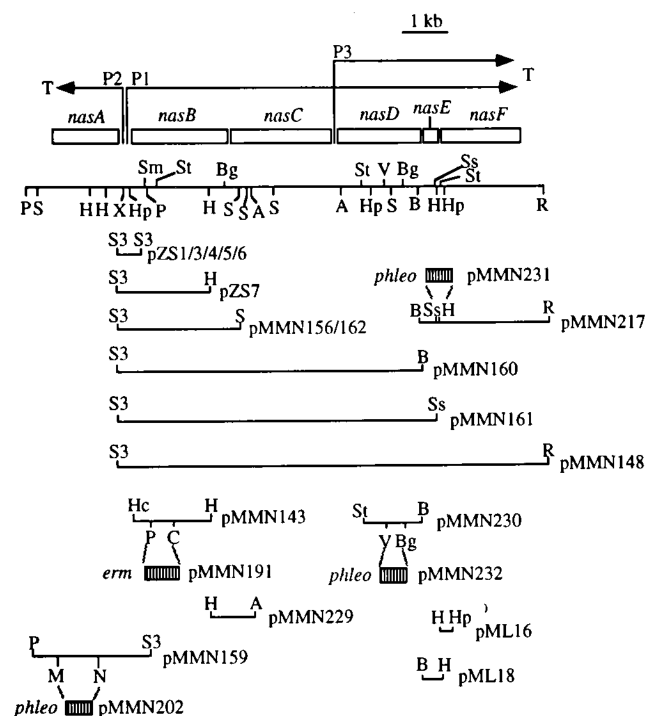

FIG. 1. Physical map of the *nasB* operon and the *nasA* gene. A restriction enzyme map of 11.5 kb of the *nasA*-to-*nasF* region is shown. Restriction site abbreviations: A, *Acc*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; V, *Eco*RV; Hc, *Hin*cII; H, *Hin*dIII; Hp, *Hpa*I; M, *Mlu*I; N, *Nco*I; P, *Pst*I; R, *Eco*RI; S, *Sph*I; S3, *Sau*3AI; Sm, *Sma*I; Ss, *Sst*I; St, *Stu*I; X, *Xba*I. Only relevant *Cla*I, *Hin*cII, *Mlu*I, *Nco*I, and *Sau*3AI recognition sites are included. The open boxes represent open reading frames that encode NasA, -B, -C, -D, -E, and -F. The locations of the putative promoters and the transcription terminators are shown by P and T, respectively. The arrows represent the directions and lengths of the transcription units. The lines below the restriction enzyme map indicate cloned segments in the corresponding plasmids shown next to each line. The hatched boxes represent phleomycin resistance (*phleo*) and erythromycin resistance (*erm*) genes used for replacement of the *nas* genes.

might be a nitrite reductase. This is confirmed by the growth phenotype and the enzyme activities of the mutant strains (see below). *E. coli* nitrite reductase consists of two polypeptides, NirB and NirD (9, 23). The C termini of nitrite reductases from *A. nidulans* and *K. pneumoniae* are both homologous to the NirD polypeptide (13). *B. subtilis* NasD lacks this C-terminal region but has a separate ORF, *nasE*, whose product has similarity to NirD (Table 2). Therefore, it is possible that NasD and NasE function as subunits of nitrite reductase in *B. subtilis.*

The N terminus of NasF is homologous to the C terminus of *E. coli* CysG (23, 26) and to the *Pseudomonas denitrificans* (5) and *Bacillus megaterium* (24) CobA proteins, which are *S*-adenosyl-L-methionine:uroporphyrinogen III methyltransferases (Table 2) catalyzing the conversion of uroporphyrinogen III to sirohydrochlorin, a common precursor for cobalmin (vitamin B12) and siroheme, a cofactor for nitrite reductases. These data suggest that NasD, NasE, and NasF are involved in nitrite reduction.

NasC is homologous to the *Synechococcus* assimilatory nitrate reductase (NarB [22]) as well as to *E. coli* formate dehydrogenase H (FdhF) (30) (Table 2). These enzymes contain the molybdopterin guanine dinucleotide-binding domain, and the binding sites for iron heme, FAD, and NADH are also found in assimilatory nitrate reductases from fungi and plants (3). But NasC, while bearing a molybdopterin guanine dinucleotide-binding domain, lacks apparent FAD- and NADH-binding domains. NasB was shown to contain motifs resembling FAD- and NADH-binding domains of nitrite reductase encoded by *nasD* as discussed above. Thus, it is likely that NasB is the electron transfer subunit and NasC is the catalytic subunit of the nitrate reductase. Similar organization was found in *K. pneumoniae* assimilatory nitrate reductase (14) and in the *E. coli* respiratory enzyme (1).

The function of the *nasA* gene product is not known, but data suggest that it may be a nitrate transporter. First, the *nasA* gene product does not show any similarity to the electron transfer or catalytic components of nitrate/nitrite reductases. Secondly, NasA is unlikely to encode a regulatory protein since *nasA* mutants did not show altered *nasB* operon expression (20). Third, the hydropathy profile of NasA suggests that it is an integral membrane protein (data not shown). Finally, as shown below, the *nasA* mutant is defective in nitrate assimilation (Table 3) but possesses high nitrate reductase activity (Table 4). A significant degree of similarity over a short region between NasA and NarK (21), the *E. coli* nitrate transporter, has been detected.

To elucidate the role of the *nas* genes in nitrate/nitrite assimilation, cells of mutant and wild-type strains were examined for growth in glucose minimal medium (MMG) (20) supplemented with either nitrate, nitrite, or ammonium (Table 3). LAB1727, the *nasB* deletion mutant, was constructed by replacement of an internal region of *nasB* in pMMN143 with an erythromycin resistance (Erm^r) gene from pJDC9 (4). The

TABLE 1. *B. subtilis* strains used, genotypes, and sources

Strain	Genotype	Source
CU636	*trpC2 narB1*	S. Zahler
JH642	*trpC2 pheA1*	J. Hoch
LAB998	*trpC2 pheA1 nasAB*::*pZS4*	This paper
LAB1166	*trpC2 pheA1* SPβ*c2del2*::Tn*917*::pZS5	This paper
LAB1167	*trpC2 pheA1* SPβ*c2del2*::Tn*917*::pZS6	This paper
LAB1727	*trpC2 pheA1* Δ*nasB*::pMMN191	This paper
LAB1798	*trpC2 pheA1* Δ*nasA*::pMMN202	This paper
LAB1889	*trpC2 pheA1 nasBC*::pMMN229	This paper
LAB1890	*trpC2 pheA1* Δ*nasB*::pMMN191 *nasBC*::pMMN229	This paper
LAB1926	*trpC2 pheA1 nasEF*::pML16	This paper
LAB1932	*trpC2 pheA1* Δ*nasE*::pMMN231	This paper
LAB1943	*trpC2 pheA1* Δ*nasE*::pMMN231 *nasEF*::pML16	This paper
LAB1965	*trpC2 pheA1 nasDE*::pML18	This paper
LAB1972	*trpC2 pheA1* Δ*nasD*::pMMN232	This paper
LAB1982	*trpC2 pheA1* Δ*nasD*::pMMN232 *nasDE*::pML18	This paper

TABLE 2. Sequence homologies of the *nasA* to *nasF* products

Gene	Probable function of product	Closest homolog(s) (% identity)
nasA	Nitrate transporter	NarK (24%) from *E. coli*
nasB	Electron transfer subunit of nitrate reductase	NirB (30%) from *E. coli*, NasB (27%) from *K. pneumoniae*, NiiA (27%) from *A. nidulans*
nasC	Catalytic subunit of nitrate reductase	NarB (31%) from *Synechococcus* sp., FdhF (28%) from *E. coli*
nasD	Subunit of nitrite reductase	NirB (33%) from *E. coli*, NasB (33%) from *K. pneumoniae*, NiiA (33%) from *A. nidulans*
nasE	Subunit of nitrite reductase	NirD (30%) from *E. coli*
nasF	*S*-Adenosyl-L-methionine:uroporphyrinogen III methyltransferase	CysG (40%) from *E. coli*, CobA (39%) from *P. denitrificans*, CobA (52%) from *B. megaterium*

resultant plasmid (pMMN191) was introduced into JH642, and an Erm^r Cm^s transformant (LAB1727) generated by double-crossover recombination was obtained. The *nasA* deletion mutant (LAB1798) was constructed in a similar way by transformation of pMMN202 (Fig. 1) into JH642. The wild-type strain, JH642, grew in all the media. Cells of the Δ*nasB* strain (LAB1727) and the Δ*nasA* strain (LAB1798) grew in ammonium and nitrite medium but not in nitrate medium. The growth defect of the *nasA* mutant in nitrate medium only is explained if *nasA* is involved in nitrate transport. The phenotype of *nasB* suggests one of two possibilities: (i) that *nasB* is required for nitrate assimilation and not for nitrite assimilation or (ii) that *nasB* as well as *nasD* encodes nitrite reductase, since both gene products show similarity to nitrite reductase as described above and the *nasB* deletion does not affect nitrite assimilation because of the redundancy of the gene. If the latter is the case, the *nasB* deletion may affect nitrate assimilation because of the polar effect on the expression of *nasC*, which encodes the catalytic subunit of nitrate reductase. This polar effect is unlikely to extend to *nasD*, since Δ*nasB* did not affect nitrite assimilation. In either case, the result that the *nasB* mutation did not lead to the defect in nitrite assimilation suggests the existence of an internal promoter (P3) before *nasD*. Evidence that *nasB* is required for nitrate assimilation was derived from the following experiment. The 0.9-kb fragment of pMMN160 containing the 3′ end of *nasB* and the 5′ end of *nasC* was inserted into pDH88 (28), which carries the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible P*spac* promoter. The resulting plasmid, pMMN229 (Fig. 1), was introduced by transformation and Campbell integration into JH642 cells. The transformant (LAB1889) bears the *nasC* gene under control of the P*spac* promoter. LAB1889 could utilize nitrite but not nitrate in the absence of IPTG, indicating that *nasC* is required for nitrate assimilation (Table 3). In the presence of IPTG, the strain was able to use nitrate as the sole nitrogen source. In contrast, LAB1890 (Δ*nasB* P*spac-nasC*) did not grow in MMG-nitrate in the presence or absence of IPTG. This strongly argues that *nasB* is involved in nitrate assimilation, since *nasC* is transcribed by the P*spac* promoter in LAB1890. It was thus concluded that *nasB* and *nasC* are required for nitrate assimilation.

A *nasD* deletion mutant (LAB1972) and a *nasE* deletion mutant (LAB1932) were constructed by transformation of JH642 with pMMN232 and pMMN231 (Fig. 1). Plasmid derivatives of pDH88, pML18 and pML16, were constructed in order to generate two strains, one having both *nasE* and *nasF* genes under P*spac* control and the other having only the *nasF* gene under P*spac* control (Fig. 1). pML18 was used to transform JH642 and LAB1972 (Δ*nasD*), while pML16 was used to transform JH642 and LAB1932 (Δ*nasE*); both plasmids recombined at the *nas* locus by Campbell integration. Table 3 shows that the *nasD* deletion mutant (LAB1972) could not assimilate nitrate or nitrite. LAB1965 (P*spac-nasEF*) assimilates nitrate and nitrite only in the presence of IPTG. LAB1982 (Δ*nasD* P*spac-nasEF*) did not grow in MMG-nitrate or MMG-nitrite in the presence of IPTG. Thus, both *nasD* and *nasE* were shown to be required for nitrite assimilation. The requirement for *nasF* in nitrite assimilation was shown by a similar experiment with the Δ*nasE* mutation and the P*spac-nasF* construct (LAB1926, -1932, and -1943 in Table 3). Growth phenotypes of

TABLE 3. Growth phenotypes of *nas* mutant strains

Strain	Relevant genotype	Growth[a] in MMG as nitrogen source with:					
		No extra source	NH_4	NO_3	NO_3[b]	NO_2	NO_2[b]
JH642	Wild type	−	+	+		+	
CU636	*narB1*	−	+	−		+	
LAB1798	Δ*nasA*	−	+	−		+	
LAB1727	Δ*nasB*	−	+	−	−	+	+
LAB1889	P*spac-nasC*	−	+	−	+	+	+
LAB1890	Δ*nasB* P*spac-nasC*	−	+	−	−	+	+
LAB1972	Δ*nasD*	−	+	−	−	−	−
LAB1965	P*spac-nasEF*	−	+	−	+	−	+
LAB1982	Δ*nasD* P*spac-nasEF*	−	+	−	−	−	−
LAB1932	Δ*nasE*	−	+	−	−	−	−
LAB1926	P*spac-nasF*	−	+	−	+	−	+
LAB1943	Δ*nasE* P*spac-nasF*	−	+	−	−	−	−

[a] Growth was monitored after 2 days of incubation at 37°C in MMG (20) liquid with or without various nitrogen sources (0.2%) as indicated. Growth was measured as absorbance at 600 nm. Absorbance similar to that of cells cultured without a nitrogen source was scored as minus and to that of cells cultured with ammonium was scored as plus.

[b] Cells were grown in the presence of 1 mM IPTG.

TABLE 4. Nitrate reductase activity in *B. subtilis* cells grown under conditions of limiting nitrogen[a]

Strain	Relevant genotype	Nitrate reductase sp act (nmol of nitrite formed/mg of protein · min) with:	
		Glutamate + KNO_3	Glutamate
JH642	Wild type	2.7	1.3
CU636	*narB1*	<0.01	<0.01
LAB1727	Δ*nasB*	<0.01	<0.01
LAB1798	Δ*nasA*	14.1	8.9
LAB1972	Δ*nasD*	5.0	1.8

[a] Cells grown overnight in MMG medium (20) supplemented with 0.2% glutamate as the sole source of nitrogen were transferred to the same medium or to the same medium supplemented with 0.01% KNO_3. Cells were harvested at mid-log phase and were washed with 0.2 M MOPS (morpholinepropanesulfonic acid) buffer (pH 7.0). Nitrate reductase activity was assayed in permeabilized cells with toluene by the standard method with dithionite-reduced methyl viologen as the reductant (25). Blanks in which the reaction was stopped at zero time were used to calculate net activities. Nitrite content was measured colorimetrically by addition of diazo-coupling reagents. Protein concentrations were assayed with a BioRad protein assay kit with bovine serum albumin as the standard. Data are averages of two to three assays, and standard deviations are within 20%.

the mutant strains described above support the conclusion from amino acid homology analysis that *nasB* and *nasC* are subunits of nitrate reductase and that *nasD*, *-E*, and *-F* are required for the activity of nitrite reductase.

Reduced methyl viologen nitrate/nitrite reductase activities were measured (25) in toluene-permeabilized cells cultured in MMG-glutamate (0.2%) and MMG-glutamate (0.2%)–KNO_3 (0.01%) (Table 4); these conditions promote high-level *nasB* expression (20). JH642 (wild type), LAB1798 (Δ*nasA*), and LAB1972 (Δ*nasD*) have nitrate reductase activities, but no activities were detected in CU636 (*narB1*) and LAB1727 (Δ*nasB*). Nitrate reductase activity is two to three times higher in cells grown in the presence of nitrate and glutamate than in those cultured in MMG-glutamate. There is no explanation at this time why significantly high nitrate reductase activity was detected in the *nasA* mutant. Even though we do not have reproducible results of nitrite reductase activity at present, this result supports the idea, based on primary structure analysis, that the *nasB* gene encodes the subunit of nitrate reductase and the *nasD* gene product is the subunit of nitrite reductase.

These genomic mapping, sequence, and mutational analyses strongly suggest that the *nas* genes contain the site of *narB1*, originally identified as the site of a mutation conferring a growth defect when nitrate is present as sole nitrogen source (29). To confirm this, CU636 (*narB1*) cells were transformed with plasmids containing *nas* DNA. The transformants were tested for growth on MMG-nitrate. Plasmid pMMN161, carrying the promoter and *nasB* to *nasD*, restored nitrate-assimilating activity to a *narB1* mutant when integrated into the *nas* region. pMMN159, carrying the promoter and *nasA*, and pMMN156 and -162, carrying the promoter and *nasB*, were unable to generate Nar^+ transformants in a *narB1* mutant recipient. On the basis of these results, the *narB1* mutation appears to be located either in *nasC* or in *nasD*. CU636 grows in MMG-nitrite (Table 3) and lacks nitrate reductase activity (Table 4). These data, taken together, seem to indicate that the *narB1* mutation is located in the *nasC* gene, although we can not completely eliminate the possibility that the *narB1* mutation is in *nasB* and is polar on *nasC* expression.

We have reported here the isolation of genes required for nitrate/nitrite assimilation in *B. subtilis*. We have previously shown that *B. subtilis* is able to grow anaerobically in the presence of nitrate and that the *nasB* mutant which contains respiratory nitrate reductase activity still grows anaerobically (8). Thus, nitrate reductase encoded by *nasB* and *nasC* seems to function solely as an assimilatory enzyme.

Nucleotide sequence accession number. The sequence used in this paper has been assigned GenBank-EMBL-DDBJ accession number D30689.

We thank Stanley Zahler for the gift of the CU636 strain and Susan Fisher for the gift of plasmid. We also thank Linc Sonenshein and David Clark for their valuable advice.

The research reported here was supported by a grant-in-aid for creative research (Human Genome Program) from the Ministry of Education, Science and Culture of Japan (to K.Y.) and grant GM45898 (to P.Z.) from the National Institutes of Health and funding from the Center for Excellence in Cancer Research, Education, and Treatment (Louisiana State University Medical Center—Shreveport).

REFERENCES

1. **Blasco, F., C. Iobbi, G. Giordano, M. Chippaux, and V. Bonnefoy.** 1989. Nitrate reductase of *Escherichia coli*: completion of the nucleotide sequence of the *nar* operon and reassessment of the role of the α and β subunits in iron binding and electron transfer. Mol. Gen. Genet. **218:**249–256.
2. **Bohin, J.-P., A. Bohin, and P. Schaeffer.** 1976. Increased nitrate reductase A activity as a sign of membrane alteration in early blocked asporogenous mutants of *Bacillus subtilis*. Biochimie **58:**99–108.
3. **Campbell, W. H., and J. R. Kinghorn.** 1990. Functional domains of assimilatory nitrate reductases and nitrite reductases. Trends Biochem. Sci. **15:**315–319.
4. **Chen, J., and D. A. Morrison.** 1987. Cloning of *Streptococcus pneumoniae* DNA fragments in *Escherichia coli* requires vectors protected by strong transcriptional terminators. Gene **55:**179–187.
5. **Crouzet, J., L. Cauchois, F. Blanche, L. Debussche, D. Thibaut, M.-C. Rouyez, S. Rigault, J.-F. Mayaux, and B. Cameron.** 1990. Nucleotide sequence of a *Pseudomonas denitrificans* 5.4-kilobase DNA fragment containing five *cob* genes and identification of structural genes encoding *S*-adenosyl-L-methionine: uroporphyrinogen III methyltransferase and cobyrinic acid *a,c*-diamide synthase. J. Bacteriol. **172:**5968–5979.
6. **Exley, G. E., J. D. Colandene, and R. H. Garrett.** 1993. Molecular cloning, characterization, and nucleotide sequence of *nit-6*, the structural gene for nitrite reductase in *Neurospora crassa*. J. Bacteriol. **175:**2379–2392.
7. **Fisher, S. H.** 1993. Utilization of amino acids and other nitrogen-containing compounds, p. 221–228. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
8. **Glaser, P., A. Danchin, F. Kunst, P. Zuber, and M. M. Nakano.** 1995. Identification and isolation of a gene required for nitrate assimilation and anaerobic growth of *Bacillus subtilis*. J. Bacteriol. **177:**1112–1115.
9. **Harborne, N., L. Griffiths, S. J. W. Busby, and J. A. Cole.** 1992. Transcriptional control, translation and function of the products of the five open reading frames of the *Escherichia coli nir* operon. Mol. Microbiol. **6:**2805–2813.
10. **Johnstone, I. L., P. C. McCabe, P. Greaves, S. J. Gurr, G. E. Cole, M. A. D. Brow, S. E. Unkles, A. J. Clutterbuck, J. R. Kinghorn, and M. A. Innis.** 1990. Isolation and characterization of the *crnA-niiA-niaD* gene cluster for nitrate assimilation in *Aspergillus nidulans*. Gene **90:**181–192.
11. **Kenny, T. J., and C. P. Moran, Jr.** 1991. Genetic evidence for interaction of σ^A with two promoters in *Bacillus subtilis*. J. Bacteriol. **173:**3282–3290.
12. **Lepesant-Kejzlarovà, J., J.-A. Lepesant, J. Walle, A. Billault, and R. Dedonder.** 1975. Revision of the linkage map of *Bacillus subtilis* 168: indications for circularity of the chromosome. J. Bacteriol. **121:**823–834.
13. **Lin, J. T., B. S. Goldman, and V. Stewart.** 1993. Structures of genes *nasA* and *nasB*, encoding assimilatory nitrate and nitrite reductases in *Klebsiella pneumoniae* M5a1. J. Bacteriol. **175:**2370–2378.
14. **Lin, J. T., B. S. Goldman, and V. Stewart.** 1994. The *nasFEDCBA* operon for nitrate and nitrite assimilation in *Klebsiella pneumoniae* M5a1. J. Bacteriol. **176:**2551–2559.
15. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227:**1435–1441.
16. **Macdonald, H., and J. Cole.** 1985. Molecular cloning and functional analysis of the *cysG* and *nirB* genes of *Escherichia coli* K12, two closely-linked genes required for NADH-dependent nitrite reductase activity. Mol. Gen. Genet. **200:**328–334.
17. **Michel, J. F. G., M. Piechaud, and P. Shaeffer.** 1970. Constitutivité vis-a-vis du nitrate de la nitrate-réductase chez les mutants asporogènes précoces de *Bacillus subtilis*. Ann. Inst. Pasteur (Paris) **119:**711–718.
18. **Murray, M. J., L. M. Siegel, S. R. Tove, and H. Kamin.** 1974. Siroheme: a new prosthetic group participating in six-electron reduction reactions cata-

lyzed by both sulfite and nitrite reductases. Proc. Natl. Acad. Sci. USA **71:**612–616.
19. **Nakano, M. M., M. A. Marahiel, and P. Zuber.** 1988. Identification of a genetic locus required for biosynthesis of the lipopeptide antibiotic surfactin in *Bacillus subtilis*. J. Bacteriol. **170:**5662–5668.
20. **Nakano, M. M., F. Yang, P. Hardin, and P. Zuber.** 1995. Nitrogen regulation of *nasA* and the *nasB* operon, which encode genes required for nitrate assimilation in *Bacillus subtilis*. J. Bacteriol. **177:**573–579.
21. **Noji, S., T. Nohno, T. Saito, and S. Taniguchi.** 1989. The *narK* gene product participates in nitrate transport induced in *Escherichia coli* nitrate-respiring cells. FEBS Lett. **252:**139–143.
22. **Omata, T., X. Andriesse, and A. Hirano.** 1993. Identification and characterization of a gene cluster involved in nitrate transport in the cyanobacterium *Synechococcus* sp. PCC7942. Mol. Gen. Genet. **236:**193–202.
23. **Peakman, T., J. Crouzet, J. F. Mayaux, S. Busby, S. Mohan, N. Harborne, J. Wootton, R. Nicolson, and J. Cole.** 1990. Nucleotide sequence, organisation and structural analysis of the products of genes in the *nirB-cysG* region of the *Escherichia coli* K-12 chromosome. Eur. J. Biochem. **191:**315–323.
24. **Robin, C., F. Blanche, L. Cauchois, B. Cameron, M. Couder, and J. Crouzet.** 1991. Primary structure, expression in *Escherichia coli*, and properties of *S*-adenosyl-L-methionine:urophorphyrinogen III methyltransferase from *Bacillus megaterium*. J. Bacteriol. **173:**4893–4896.
25. **Stewart, V., and J. Parales, Jr.** 1988. Identification and expression of genes *narL* and *narX* of the *nar* (nitrate reductase) locus in *Escherichia coli* K-12. J. Bacteriol. **170:**1589–1597.
26. **Warren, M. J., C. A. Roessner, P. J. Santander, and A. I. Scott.** 1990. The *Escherichia coli cysG* gene encodes S-adenosylmethionine-dependent uroporphyrinogen III methylase. Biochem. J. **265:**725–729.
27. **Wierenga, R. K., M. C. H. De Maeyer, and W. G. J. Hol.** 1985. Interaction of pyrophosphate moieties with α-helixes in dinucleotide binding proteins. Biochemistry **24:**1346–1357.
28. **Yansura, D. G., and D. J. Henner.** 1984. Use of the *Escherichia coli lac* repressor and operator to control gene expression in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **81:**439–443.
29. **Zahler, S. A., L. G. Benjamin, B. S. Glatz, P. F. Winter, and B. J. Goldstein.** 1976. Genetic mapping of *alsA*, *alsR*, *thyA*, *kauA*, and *citD* markers in *Bacillus subtilis*, p. 35–43. *In* D. Schlessinger (ed.), Microbiology—1976. American Society for Microbiology, Washington, D.C.
30. **Zinoni, F., A. Birkmann, T. C. Stadman, and A. Böck.** 1986. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83:**4650–4654.
31. **Zuber, P.** 1985. Localizing the site of *spo0*-dependent regulation in the *spoVG* promoter of *Bacillus subtilis*, p. 149–156. *In* J. A. Hoch and P. Setlow (ed.), Molecular biology of microbial differentiation. American Society for Microbiology, Washington, D.C.
32. **Zuber, P.** Unpublished data.
33. **Zuber, P., and R. Losick.** 1987. Role of AbrB in Spo0A- and Spo0B-dependent utilization of a sporulation promoter in *Bacillus subtilis*. J. Bacteriol. **169:**2223–2230.