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MATHEMATICAL AND SYSTEM BIOLOGY

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Regulation of Nitrogen Metabolism in Gram-Positive Bacteria

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Abstract—A search for new members of the TnrA and GlnR regulons, responsible for assimilation of nitrogen in Gram-positive bacteria, was performed. Common regulatory signals with consensus sequences ATGTNAWWWWWWWTNACAT and TGTNAWWWWWWWTNACA were identified for GlnR and TnrA, respectively. The structure was described and new potential members were found in *Bacillus subtilis, B. licheniformis, Geobacillus kaustophilus*, and *Oceanobacillus iheyensis* for the TnrA/GlnR regulons; in *B. halodurans* for the TnrA regulon; and in *Lactococcus lactis, Lactobacillus plantarum, Streptococcus pyogenes, S. pneumoniae, S. mutans, S. agalactiae, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus*, and *St. epidermidis* for the GlnR regulon.

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Key words: TnrA regulon, GlnR regulon, *Bacillus subtilis*, assimilation of nitrogen

INTRODUCTION

It is known that synthesis of nucleic acids, proteins, and other nitrogen-containing molecules in the cytoplasm requires the presence of amino groups, which are integrated in molecules by direct amination or transamination. Inorganic nitrogen (nitrate, nitrite, molecular nitrogen) is preliminarily reduced to ammonia (assimilation reduction of nitrogen) and, only after that, is included in organic compounds. In addition, ammonium ions (NH_4^+) can be generated during catabolism of purines and urea. Some bacteria can utilize purine bases as a nitrogen source. In this case, a metabolic pathway functions in which guanine and hypoxanthine degrade to xanthine with liberation of ammonia. Xanthine further degrades to urea. Urease catalyzes urea reduction to ammonium. Incorporation of ammonium ions is catalyzed by glutamine synthase (glutamine synthesis) or glutamate synthase (glutamate synthesis). Other compounds with amino groups are synthesized by transamination of primary amino acids, glutamine and glutamic acid.

The metabolic pathways of nitrogen assimilation differ among diverse species of microorganisms. Thus, in *E. coli* and other Gram-negative bacteria, genes involved in nitrogen metabolism are activated by the two-component Ntr system [1], consisting of the products of the *gln*L (NtrB) and *gln*G (NtrC) genes [2].

An analog of the Ntr system has not been found in Gram-positive microorganisms. Among bacteria of this group soil, sporulating bacterium *Bacillus subtilis* has been best characterized with respect to nitrogen metabolism. The assimilation of nitrogen in *B. subtilis* is controlled by two transcription factors, GlnR and TnrA, which belong to the MerR family of DNA-binding regulatory proteins [3, 4]. GlnR regulates the expression of the *gln*RA operon, which encodes glutamine synthase (GlnA). Under conditions of a nitrogen excess, GlnR functions as a repressor of the *gln*RA operon [5]. In contrast to GlnR, TnrA may repress as well as activate transcription. Under conditions of an excess of glutamine and glutamate in the cell, TnrA binds to glutamine synthase, forming an inactive complex. As a result, TnrA cannot interact with specific operators and regulate gene expression. Under conditions of a lack of glutamine and glutamate, TnrA is released from the complex GlnA–TnrA, binds to specific operators, and regulates transcription. Nitrogen metabolism genes can also be regulated by other transcriptional factors, for instance, by the global regulatory protein CodY in *B. subtilis* [5].

There are 17 known transcriptional units (genes and operons) whose regulatory regions contain the specific TnrA-binding site, which is a palindrome with consensus TGTNANAWWWTMTNACA [5–10]. TnrA activates transcription of the *gab*P (γ-aminobutyrate transport), *nas*A (nitrate transporter), *nas*B (nitrate reductase), *nas*DEF (nitrite reductase), *nrg*AB (ammonium transporter), and *ure*ABC (urease) operons and the *puc* genes (catabolism of purines), and represses the *gln*RA (glutamine synthase), *glt*AB (glutamate synthase), and *als*T (alanine transporter) operons. Moreover, TnrA activates the expression of its own gene, *tnr*A [4].

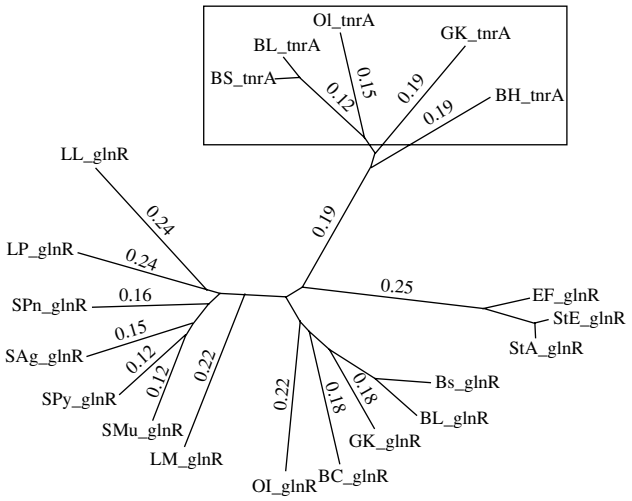

Fig. 1. Phylogenetic tree of TnrA and GlnR homologs of bacilli, *Listeria*, staphylococci, and streptococci. Designations: BS, *Bacillus subtilis*; BL, *B. licheniformis*; BC, *B. cereus*; BH, *B. halodurans*; GK, *Geobacillus kaustophilus*; OI, *Oceanobacillus iheyensis*; EF, *Enterococcus faecalis*; LM, *Listeria monocytogenes*; StA, *Staphylococcus aureus*; StE, *St. epidermidis*; LP, *Lactobacillus plantarum*; LL, *Lactococcus lactis*; Sag, *Streptococcus agalactiae*; SMu, *S. mutans*; SPn, *S. pneumoniae*; and Spy, *S. pyogenes*.

An ortholog of *B. subtilis* transcriptional regulator GlnR has been found in Gram-positive bacteria *Lactobacillus* spp., *Staphylococcus aureus*, and *Streptococcus* spp. In all these cases GlnR represses the expression of the glutamine synthase gene (*gln*A) [11–13].

The aim of this work was to analyze the TnrA and GlnR regulons, responsible for assimilation of nitrogen, in Gram-positive bacteria in order to reconstruct the regulation of this metabolic system and to study its conservation among diverse species of bacteria.

EXPERIMENTAL

We analyzed the genomic nucleotide sequences of Gram-positive bacteria *B. subtilis, B. licheniformis, B. cereus, B. halodurans, Geobacillus kaustophilus, Oceanobacillus iheyensis, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, St. epidermidis, Lactobacillus plantarum, Lactococcus lactis, Streptococcus agalactiae, S. mutans, S. pneumoniae*, and *S. pyogenes*. The sequences were obtained from GenBank [14].

Protein sequence alignments were constructed using the ClustalW program [15]. Regulatory sites were identified and a training set was constructed using the Genome Explorer and SignalX programs [16].

A candidate gene was assigned to the TnrA regulon when the gene (or the corresponding operon) contained a putative GlnA-binding site with a weight higher than the threshold 4.50 in region –300 to +50 bp relative to the translation start.

A candidate gene was assigned to the TnrR regulon when the gene (or the corresponding operon) contained a putative GlnR-binding site with a weight higher than the threshold 5.40 in region –300 to +50 bp relative to the translation start. A stricter threshold was used to search for genes of the GlnR regulon because the GlnR signal was 2 bp longer than the TnrA signal.

When the operon structure was unknown, genes transcribed in the same direction and separated by no more than 50 bp were considered to belong to one operon.

The genes were designated according to the corresponding *B. subtilis* orthologs.

RESULTS AND DISCUSSION

The search for orthologs of the regulatory genes *tnr*A and *gln*R revealed a lack of one of the regulators of nitrogen assimilation in some bacterial genomes. For instance, *B. cereus, Listeria monocytogenes, Lactobacillus plantarum, Staphylococcus* spp., and *Streptococcus* spp. have only *gln*R. *Bacillus halodurans* possesses only *tnr*A and lacks *gln*R. Other bacilli have both *tnr*A and *gln*R.

A phylogenetic tree was constructed for TnrA and GlnR of all organisms analyzed (Fig. 1). The tree has four branches. The GlnR proteins of staphylococci form the first branch. The second branch contains GlnR of *Streptococcus* and *Listeria* spp. The two other branches are formed by bacillar TnrA and GlnR. Notably, the bacillar TnrA branch is well separated from the branches of GlnR from bacilli and other Gram-positive microorganisms.

The TnrA signal could not be identified in the presence of both regulators because of its high similarity to the GlnR signal (Fig. 2). Therefore, in the genomes containing both regulators, a search for members of the TnrA/GlnR regulon was performed using the signal for TnrA because this regulator is global and the corresponding recognition rule is less strict.

Genes of the TnrA/GlnR Regulon

A training set for constructing a signal included 18 regulatory regions of the known genes of the *B. subtilis* TnrA/GlnR regulon whose products are involved in nitrogen assimilation. Application of the signal recognition procedure to this set allowed us to identify a common regulatory signal with consensus TGTNAWWWWWWTNACA. The signal was used to search for new genes potentially belonging to the TnrA/GlnR regulon in all organisms under study.

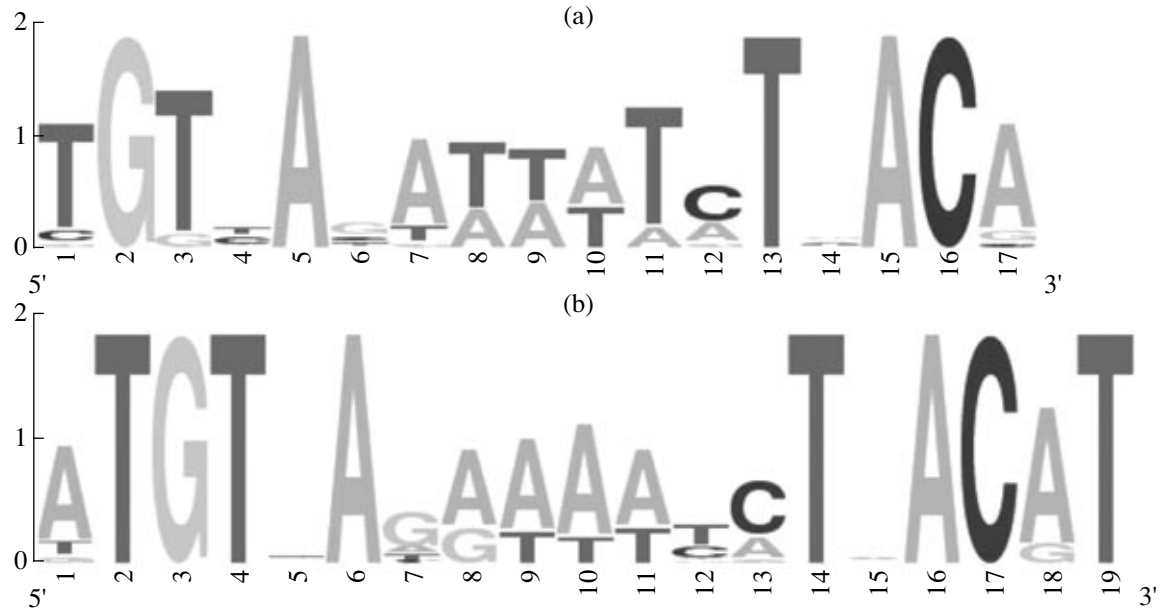

Fig. 2. Pictorial representation of the regulatory signals for (a) TnrA and (b) GlnR of Gram-positive bacteria [17].

Candidate genes were revealed in each genome, including 44 in *B. subtilis*, 36 in *B. licheniformis*, 18 in *O. iheyensis*, and 10 in *G. kaustophilus*. Then pairwise comparisons were performed with all orthologous genes of all microorganisms. As a result, we revealed 12 operons that had the conserved TnrA/GlnR site upstream of orthologous genes in at least two genomes. Another 11 operons lacked the conserved TnrA-binding site, but their products were involved in nitrogen assimilation (Fig. 3).

In all genomes analyzed, the conserved TnrA-binding site was observed upstream of the *nrg*AB operon. In *B. licheniformis* we revealed two *nrg*AB operons with potential sites for TnrA.

In all bacilli, we found orthologs of the *gln*RA operon with a reliable TnrA site in the regulatory region (*O. iheyensis*), with a weak site (*B. subtilis* and *B. licheniformis*), or without it (*G. kaustophilus*).

The *nas* operon with the conserved TnrA site occurs in the genomes of all bacilli with the exception *O. iheyensis*, although with several differences in structure. In *B. subtilis*, the *nas* locus contains two TnrA sites: one is between oppositely directed *nas*A and *nas*B, and the other is upstream of *nas*D.

Orthologs of *als*T gene were found in all bacteria except *G. kaustophilus.* However, reliable TnrA sites were revealed only in *B. subtilis* and *B. licheniformis*.

The *glt*AB operon, coding for glutamate synthase, exists in the genomes of all bacilli examined, although the TnrA-dependent regulation is preserved only in *B. subtilis* and *B. licheniformis*.

In *B. subtilis*, the *puc* operon, required for catabolism of purines, presumably contains two binding sites for TnrA. Separate genes of this operon were found in *B. licheniformis*, but they are not regulated by TnrA. The other bacilli lack purine catabolism genes.

Orthologs of the genes encoding the ATP-depended transporter of oligopeptides (*opp*ABCDF) with TnrA sites were found in *B. subtilis* and *B. licheniformis*. The TnrA site was not found in the *O. iheyensis opp*ABCDF operon, and an ortholog of this operon was not revealed in *G. kaustophilus*.

The *yod*F gene, whose product is homologous to the proline transporter, is contained in the genomes of *B. subtilis* and *B. licheniformis*, and in both cases, the TnrA site is conserved in the regulatory region. In other bacteria, an ortholog of this gene was not revealed.

The TnrA-regulated genes *ywr*D (γ-glutamyltransferase) and *gln*Q (glutamine transporter) were found only in *B. subtilis*. The orthologs of these genes are not regulated by TnrA in other bacilli.

TnrA/GlnR-regulated *gab*P, coding for the γ-aminobutyrate transporter, was found only in *B. subtilis*; its ortholog was not found in other bacilli. The *arg*C gene, involved in arginine biosynthesis, was revealed in all bacilli, but its regulation seems to be conserved only in *B. subtilis* and *B. licheniformis*.

The gene of the branched amino acids transporter (*bra*B) has a potential TnrA site in *B. subtilis* and *B. licheniformis*. Its ortholog from *O. iheyensis* does

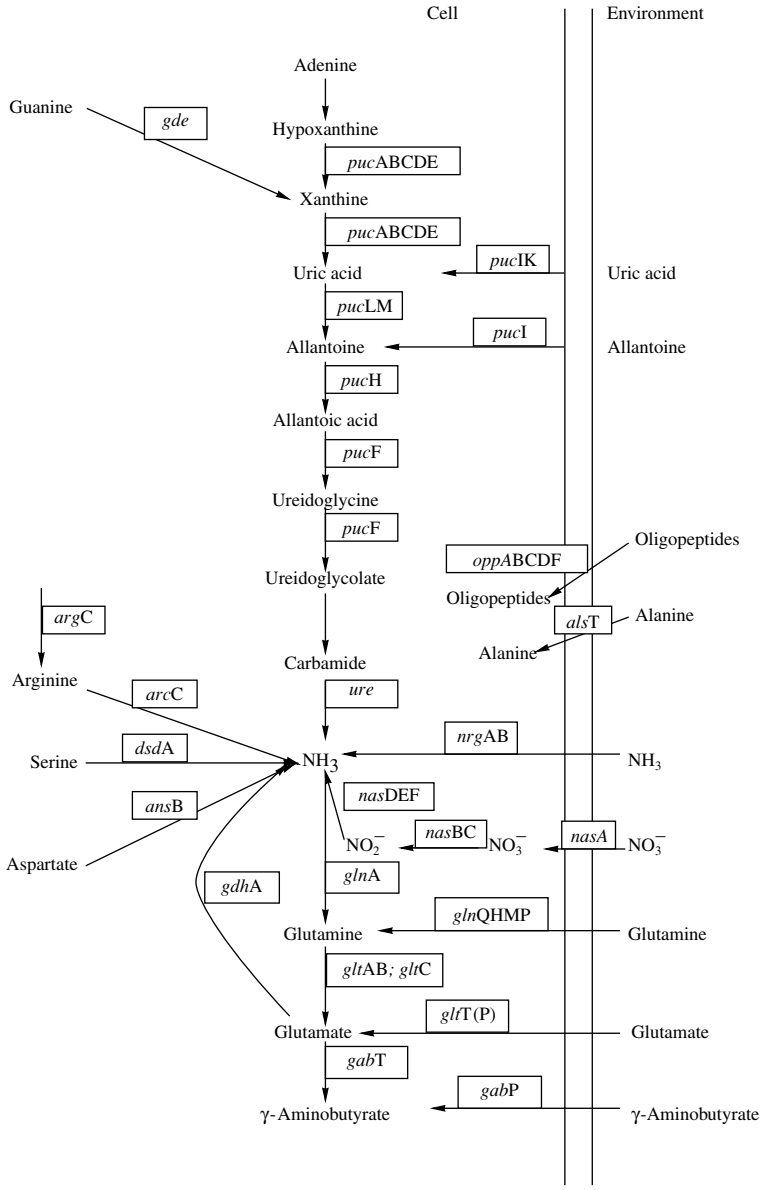

Fig. 3. Pathway of nitrogen metabolism in *Bacillus subtilis*.

not preserve the TnrA regulation. Orthologs of this gene were not found in other bacilli (Table 1).

Genes of the TnrA Regulon

Among the genomes analyzed, only the *B. halodurans* genome possesses a single TnrA regulator of nitrogen assimilation (Table 1). The search for genes of the TnrA regulon was performed using the same approach as in bacilli with two regulators. We found that *B. halodurans* has much in common with other bacilli. Moreover, in *B. halodurans*, the regulation of the ammonium transporter gene (*nrg*A), the *nas* operon, the alanine transporter gene (*als*T), and the purine catabolism genes (*puc*) is preserved. The regulation of genes for glutamate synthase (*glt*AB) and glutamate dehydrogenase (*roc*G) seems not to be preserved. *B. halodurans* lacks *gln*R but possesses two paralogs of *gln*A, both with significant TnrA-binding sites. The glutamate transporter gene (*glt*T) was revealed in all bacilli, but proved to be regulated by TnrA only in *B. halodurans*. In *B. halodurans*, but not in *B. subtilis* and *G. kaustophilus*, the urease operon *ure*ABC has a TnrA-binding site. Orthologs of *ade*C, coding for adenine deaminase, were found in all bacilli examined; however, the TnrA-dependent regulation was revealed only in *B. halodurans*. The *yfl*A gene of the amino acid transporter is regulated by TnrA in *B. halodurans*, but probably not in other bacilli.

Table 1. TnrA/GlnR regulon in bacilli

Bacillus subtilis	*Bacillus licheniformis*	*Bacillus cereus**	*Bacillus halodurans***	*Oceanobacillus iheyensis*	*Geobacillus kaustophilus*	Function
&*tnrA*	&*tnrA*	–	&*tnrA*	&*tnrA*	&*tnrA*	Regulator of transcription
&*nrgAB*	&*nrgAB1*; &*nrgBA2*	&*nrgA*	&*nrgA*	&*nrgAB*	&*nrgA*	Ammonium transporter
&*glnRA*	&*glnRA*	&*GlnR*	&*glnA1*, &*glnR2*	&*glnRA*	&*glnRA*	Glutamine synthase
nasA⟨&⟩*nasBC*&*nasDEF*	&*nasBC-nasDEF*	*nasED*	*nasADEC*&*nasF*	–		Nitrate/nitrite reductase
&*alsT*	&*alsT*	&*alsT*	&*alsT*	*alsT*	–	Alanine transporter
&*gltAB gltC*	*gltAB*⟨&⟩*gltC*	*gltA*	*gltAB*	*gltAB*	*gltAB*⟨ ⟩*gltC*	Glutamate synthase
pucH⟨&⟩*pucR-*&*pucJKLM*	*pucH-pucR*	–	*pucH-*&*pucR-pucLM*	–	–	Purine catabolism
&*oppABCDF*	&*oppA*	&*oppBCDF*	*oppA*⟨&⟩*oppCBDF*	–	–	Oligopeptide transporter
yodF⟨&⟩*yodE*	&*yodF*	–	–	–	–	Proline transporter homolog
&*yycCB*	&*yycCB*	–	–	*yycCB*	*yycCB*	ABC transporter
&*gabP*	–	*gabP*	–	–	–	γ-Aminobutyrate transporter
&*argC*	&*argC*	–	*argC*	*argC*	*argC*	Arginine biosynthesis
&*braB*	&*braB*	*braB*	–	*braB*	–	Branched amino acid transporter
&*ywrD*	*ywrD*	–	–	*ywrD*	*ywrD*	γ-Glutamyltransferase homolog
&*glnQHMP*	–	–	–	*glnQHMP*	*glnQHMP*	Glutamine ABC transporter
gltT; *gltP*	*gltT*; *gltP*	&*gltP*	*gltT*	*gltT*	*gltT*	Glutamate transporter
ureABC	–	–	&*ureABCEFGD*	–	*ureABCEFGDH*	Urease
adeC	*adeC*	*adeC*	&*adeC*	*adeC*	*adeC*	Adenine deaminase
YflA	*yflA*	*yflA*	*yflA*	–	*yflA*	Amino acid transporter
–	&*rocG*	–	*rocG*	–	–	Glutamate dehydrogenase
nagAB	*nagAB nagAB*	*nagAB*	&*nagAB*	*nagA*; *nagB*	*nagAB*	Catabolism of acetyl-D-glucosamine

Note: Designations: (&⟩ presence of a binding site; (&) weak TnrA-binding site with a weight of 4.0–4.5; (⟨ ⟩) lack of the TnrA-binding site.

* *B. cereus* has only GlnR.

** *B. halodurans* has only TnrA.

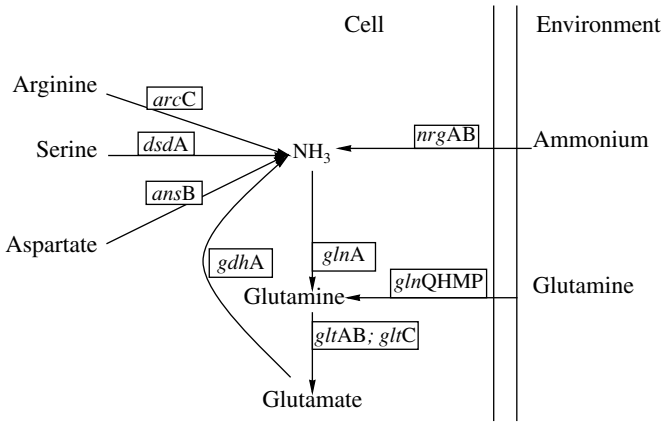

Fig. 4. Nitrogen assimilation regulated by GlnR in *Streptococcus*, *Staphylococcus, Lactococcus*, and *Listeria* spp.

Genes of the GlnR Regulon

The expression of the *gln*RA operon is regulated by GlnR in various bacteria. Therefore, the regulatory sites of the *gln*RA operon from all bacteria were included in a training set for constructing the signal. Application of a signal recognition procedure allowed us to identify a common regulatory site with consensus ATGTNAWWWWWWTNACAT, which differs from the TnrA signal in two terminal positions. In view of the high similarity of the TnrA and GlnR signals (Fig. 2), a search for new GlnR-regulated genes was performed in the genomes that code for GlnR (*Streptococcus, Lactobacillus, Staphylococcus*, and *Listeria* spp.), but not for TnrA. In each genome, genes containing a potential GlnR-binding site were selected and then compared in each pair of genomes. As a result, we revealed four operons (*gln*RA, *nrg*A, *gln*QHMP, and *gdh*A) with the GlnR sites conserved in at least two genomes and three operons (*arc*A, *dsd*A, and *ans*B) that lacked the conserved GlnR-binding sites, but whose products were involved in nitrogen assimilation (Fig. 4).

As expected, in all bacteria, the GlnR sites preceded the *gln*RA operon, coding for glutamine synthase. Moreover, different numbers of glutamine ABC transporter genes were revealed in many organisms. The primary identification of transporters was performed by homology, and then their regulation was analyzed. As a result, the GlnR-regulated transporter genes were revealed: one in *Lactococcus lactis, St. aureus, L. plantarum*, and *S. pyogenes*; two in *S. pneumoniae, S. mutans*, and *S. agalactiae*; and three in *E. faecalis*. At the same time, none of the glutamine transporter genes is regulated by GlnR in *Listeria monocytogenes* and *St. epidermidis*.

We revealed that *nrg*A, required for ammonium transport, is regulated by GlnR in *L. plantarum, Lactococcus lactis, S. mutans, Listeria monocytogenes*, and *E. faecalis* and is not regulated in *S. agalactiae*, while *S. pyogenes* and *S. pneumoniae* lack orthologs of this gene.

The *gdh*A gene, responsible for synthesis of glutamate dehydrogenase, is probably regulated by GlnR in *S. pyogenes* and *S. mutans*, but not in *L. plantarum* and *S. agalactiae*. In other bacteria, orthologs of this gene were not revealed.

The GlnR-regulated genes of serine dehydrogenase (*dsd*A) and aspartate ammonia-lyase (*ans*B) were revealed only in *L. plantarum*. The arginine deiminase gene (*arc*A) was revealed in all bacteria except *L. plantarum* and *S. mutans*, whereas the GlnR site was revealed only in *S. pneumoniae* (Table 2).

In *B. subtilis*, GlnR regulates the *gln*RA operon [5]. It is impossible to identify other GlnR-regulated genes in bacilli because the TnrA and GlnR signals are virtually identical (Fig. 2). However, *B. cereus* is worthy of special consideration. This bacterium lacks TnrA, and only GlnR controls nitrogen assimilation. The search of the *B. cereus* genome revealed that the GlnR regulon contains the *gln*RA and *nrg*A operons (as in other bacteria with GlnR-regulated nitrogen assimilation) as well as the *opp*BCDF, *als*T, and *glt*T operons, which belong to the TnrA regulon in other bacilli.

This work revealed a significant plasticity of the TnrA/GlnR regulon in bacilli. The structure of this regulon varies significantly in different bacteria. The GlnR regulon found in *Streptococcus, Listeria*, and *Staphylococcus* spp. is highly conserved, mainly containing genes of glutamine transport and utilization. In spite of this plasticity, we identified a core of the bacillar TnrA/GlnR regulon, which consists of genes found in almost all genomes and contains potential TnrA- and GlnR-binding sites in most cases. The genomes of most bacilli have genes of both duplicated regulators, TnrA and GlnR. Yet GlnR apparently regulates only the *gln*RA operon. This is evident from experimental data obtained with *B. subtilis* [5], although it cannot be verified using bioinformatics approaches because of the high similarity of the binding signals for these two factors. A coregulation by both factors cannot be excluded for several operons. In organisms with only one factor preserved (for instance, GlnR in *B. cereus*), this factor regulates most genes of the metabolic pathway, including genes that are regulated by the second factor in other genomes. Since *Streptococcus, Staphylococcus*, and *Listeria* spp. have only GlnR, the duplication of GlnR/TnrA apparently took place in a common ancestor of bacilli, and then *B. cereus* (TnrA) and *B. halodurans* (GlnR) lost one of the factors.

Table 2. GlnR-dependent regulation in *Streptococcus*, *Staphylococcus*, *Lactococcus*, and *Listeria* spp.

Enterococcus faecalis	*Lactobacillus plantarum*	*Lactococcus lactis*	*Streptococcus pyogenes*	*Streptococcus pneumoniae*	*Streptococcus mutans*	*Streptococcus agalactiae*	*Listeria monocyto-genes*	*Staphylo-coccus aureus*	*Staphylo-coccus epidermidis*	Function
&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	&*glnRA*	Glutamate synthase
&*glnQHMP*	&*glnQHMP*	&*glnQHMP*	&*glnQHMP*	&*glnQHMP*	&*glnQHMP*	&*glnQHMP*	*glnQHMP*	&*glnQHMP*	glnQHMP	Glutamine ABC transporter
&*glnQHMP*	*glnQHMP*		*glnQHMP*	*glnQHMP*	&*glnQHMP*	&*glnQHMP*	*glnQHMP*			
&*glnQHMP*	*glnQHMP*		*glnQHMP*	*glnQHMP*	*glnQHMP*	*glnQHMP*				
	glnQHMP				*glnQHMP*	*glnQHMP*				
						glnQHMP				
&*nrgA*	&*nrgA*	&*nrgA*	–	–	&*nrgA*	*nrgA*	&*nrgA*	*nrgA*	*nrgA*	Ammonium transporter
–	*gdhA*	–	–	&*gdhA*	&*gdhA*	*gdhA*	*gdhA*	*gdhA*	*gdhA*	Glutamate dehydrogenase
–	&*dsdA*	–	–	–	–	–	–	–	–	Serine dehydratase
–	&*ansB*	–	–	–	–	–	–	–	–	Aspartate ammonia-lyase
arcA	–	*arcA*	*arcA*	&*arcA*	–	*arcA*	*arcA*	*arcA*	*arcA*	Arginine deiminase

Note: (&) presence of a binding site; (&) weak TnrA-binding site with a weight of 4.5–5.4.

ACKNOWLEDGMENTS

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REFERENCES

1. Wray L.V., Zalieckas J.M., Ferson A.E., Fisher S.H. 1998. Mutational analysis of the TnrA-binding sites in the *Bacillus subtilis nrg*AB and *gap*B promoter regions. *J. Bacteriol*. **180**, 2943–2949.
2. Barrios H., Valderrama B., Morett E. 1999. Compilation analysis of σ^{54}-dependent promoter sequences. *Nucleic Acids Res*. **27**, 4305–4313.
3. Robichon D., Arnaud M., Gardan R., Pragai Z., O'Reilly M., Rapoport G., Debarbouille M. 2000. Expression of a new operon from *Bacillus subtilis, ykz*B–*yko*R, under the control of the TnrA and PhoP-PhoR global regulators. *J. Bacteriol*. **182**, 1226–1231.
4. Fisher S.H. 1999. Regulation of nitrogen metabolism in *Bacillus subtilis*: Vive la difference! *Mol. Microbiol*. **32**, 223–232.
5. Yoshida K., Yamagguchi H., Kinehara M., Ohki Y., Nakaura Y., Fujita Y. 2003. Identification of additional TnrA-regulated genes of *Bacillus subtilis* associated with a TnrA box. *Mol. Microbiol*. **49**, 157–165.
6. Belytsy B.R., Wray Jr.L., Fisher S.H., Bohannon D.E., Sonenshein A.L. 2000. Role of TnrA in nitrogen source-dependent repression of *Bacillus subtilis* glutamate synthase gene expression. *J. Bacteriol*. **182**, 5939–5947.
7. Brandenburg J.L., Wray L.V., Beier L., Jarmer H., Saxild H.H., Fisher S.H. 2002. Roles of PucR, GlnR, and TnrA in regulating expression of the *Bacillus subtilis ure*P3 promoter. *J. Bacteriol*. **184**, 6060–6064.
8. Nakano M.M., Hoffman T., Zhu Y., Jahn D. 1998. Nitrogen and oxygen regulation of *Bacillus subtilis nas*DEF encoding NADH-dependent nitrite reductase by TnrA and ResDE. *J. Bacteriol*. **180**, 5344–5350.
9. Schultz A.C., Nygaard P., Saxild H.H. 2001. Functional analysis of 14 genes that constitute the purine catabolic pathway in *Bacillus subtilis* and evidence for a novel controlled by the PucR transcription activator. *J. Bacteriol*. **183**, 3293–3302.
10. Beier L., Nygaard P., Jarmer H., Saxild H.H. 2002. Transcription analysis of the *Bacillus subtilis* PucR regulon and identification of *cis*-acting sequence required for PucR-regulated expression of genes involved in purine catabolism. *J. Bacteriol*. **184**, 3232–3241.
11. Gustafson J., Strassle A., Hachler H., Kayzer F.H., Berger-Bachi B. 1994. The *fem*C locus of *Staphylococcus aureus* required for methicillin resistance includes the glutamine synthetase operon. *J. Bacteriol*. **176**, 1460–1467.
12. Fink D., Weischuh N., Reuther J., Wohlleben W., Engels A. 2002. Two transcriptional regulators GlnR and GlnRII are involved in regulation of nitrogen metabolism in *Streptomyces coelicolor* A3(2). *Mol. Microbiol*. **46**, 331–347.
13. Varmanen P., Savijoki K., Avall S., Palva A., Tynkkynen S. 2000. X-prolyl dipeptidyl aminopeptidase gene (*pep*X) is part of the *gln*RA operon in *Lactobacillus hamnosus. J. Bacteriol*. **182**, 146–154.
14. Benson D.A., Karsch-Mizrachi I., Lipman D.J., Ostell J., Wheeler D.L. 2005. GenBank. *Nucleic Acids Res*. **33**, 34–38.
15. Lim A., Zhang L. 1999. WebPHYLIP: A web interface to PHYLIP. *Bioinformatics*. **15**, 1068–1069.
16. Mironov A.A., Vinokurova N.P., Gelfand M.S. 2000. Software for analyzing bacterial genomes. *Mol. Biol*. **34**, 253–262.
17. Crooks G.E., Hon G., Chandonia J.M., Brenner S.E. 2004. WebLogo: A sequence logo generator. *Genome Res*. **14**, 1188–1190.