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Bacillus clausii and *Bacillus halodurans* lack GlnR but Possess Two Paralogs of *glnA*

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Abstract

Bacillus clausii and *Bacillus halodurans* lack GlnR but possess a single TnrA regulator of nitrogen assimilation and two paralogs of *glnA*. *Bacillus clausii* contains two paralogs of the gene encoding the glutamine synthetase (GS), *glnA1 (ABC3940)* and *glnA2 (ABC2179)*. The *glnA1* gene contains a TnrA site. This TnrA site is located downstream of the -10 region of the promoter. However, the *glnA2* gene does not contain the TnrA site at its regulatory region. *Bacillus halodurans* possesses two paralogs of *glnA*, both with TnrA-binding sites. The *glnA1 (BH2360)* gene contains a TnrA site, which overlaps the -10 region of the *glnA1* promoter, and the *glnA2 (BH3867)* gene contains a TnrA site downstream of the -10 region of its promoter. Also, the *Bacillus subtilis* dicistronic *glnRA* operon, which encodes GlnR and GS, contains two TnrA sites (*glnRAo1* and *glnRAo2*) in its promoter region. The *glnRAo2* site, which overlaps the -35 region of the *glnRA* promoter, was shown to be required for regulation by TnrA. These results indicate that TnrA regulates the expression of GS, through binds to TnrA sites which overlaps the -35 and -10 promoter elements or lies downstream of the theirs of the *glnA* or *glnRA* promoters.

Keywords: Gene regulation; Glutamine synthetase; Transcription factors; TnrA; GlnR

Introduction

The MerR family is a group of transcriptional activators, which regulates gene expression and controls transcription in response to diverse physiological signals [1], such as nitrogen availability [2]. The GlnR and TnrA belong to the MerR family of DNA-binding regulatory proteins. This group of activators contains a conserved N-terminal DNA-binding domain that is approximately 70 amino acids in length [1,3]. The *Bacillus clausii* and *Bacillus halodurans* TnrA transcription factors have been found to contain 100 amino acids. Also, the *Bacillus subtilis* TnrA, as one of the best understood members of the MerR family, composed of 110 amino acids.

In *B. subtilis, Bacillus licheniformis, Geobacillus Kaustophilus* and *Oceanobacillus iheyensis*, the two transcription factors TnrA and GlnR control many genes for utilization of glutamine and other nitrogen-containing compounds. *Bacillus clausii* and *B. halodurans* lack GlnR but possesses a single TnrA regulator of nitrogen assimilation and two paralogs of *glnA*. Other Gram-positive bacteria, such as *Streptococcus, Listeria* and *Staphylococcus* lack TnrA but possess the highly conserved GlnR regulon, which mainly contains genes of glutamine transport and utilization [4].

The *B. subtilis* TnrA is a global regulator that responds the availability of nitrogen sources and both activates and represses many *B. subtilis* genes during nitrogen limitation. It is involved in the direct and indirect regulation of many genes, which are involved in the transport and catabolism of nitrogen-containing compounds [5-7]. The *B. subtilis* GlnR regulates the expression of the *glnRA* operon, which encodes glutamine synthase (GlnA). Under conditions of a nitrogen excess, GlnR functions as a repressor of the *glnRA* operon [4,7]. When nitrogen sources are in excess, the *B. subtilis* glutamine synthetase (GS), a key enzyme in nitrogen metabolism, becomes subject to feedback inhibition by glutamine and adenosine monophosphate (AMP). The feedback-inhibited GS forms a complex with TnrA via its C-terminal domain, thereby preventing TnrA from interacting with specific operators and regulating gene expression (6). In contrast, under conditions of nitrogen-limited growth, TnrA is released from the GS–TnrA complex and then binds to the TnrA sites of specific operators, consequently regulating transcription [3].

The TnrA of *B. subtilis,* as well as activating its own expression also activates transcription of the *gabP, nasA, nasB, nasDEF, nrgAB*, and the *puc* genes, and represses that of the *glnRA, gltAB,* and *alsT* operons [7,8]. A genome-wide analysis for TnrA-regulated genes of *B. clausii* associated with a TnrA box was shown that there were some transcription units containing a putative TnrA box, such as *tnrA, glnA, nrgA, nasFDEB,* and *puc* genes [9]. It has recently been suggested that the TnrA is also involved in expression of the extracellular alkaline protease (*aprE*), with a link existing between *aprE* expression and the *B. subtilis* GlnA-TnrA system [10]. It has previously been found that *aprE* expression increases when the GS gene, *glnA,* is disrupted. This increase in expression has been attributed to a decrease in the expression of *scoC*, which encodes a negative regulator of *aprE* expression. It has also been observed that the effect of *glnA* on *scoC* expression is abolished by the further disruption of *tnrA*, thus indicating that *aprE* expression is under global regulation through TnrA [10].

Bacillus clausii is also known to produce a commercially important extracellular alkaline serine protease (AprE) [11,12]. Elucidating of the molecular mechanisms of the metabolism and gene regulatory networks could thus be used to design metabolic engineering strategies

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for maximizing alkaline serine protease production in *B. clausii*. The aim of this work was to distinguish and analyze the potentially TnrA sites of *glnA* promoter regions of *B. clausii* and *B. halodurans*, responsible for metabolism of nitrogen, and thus have an insight into the nature of the regulation of this metabolic system and reveal the similarities and differences of the associated transcriptional regulatory networks, present in *B. clausii, B. halodurans* and *B. subtilis*.

Materials and Methodology

Bacterial strains and nucleotide sequences

The complete genome sequence of *B. clausii KSM-K16* and *B. halodurans C-125,* were obtained from GenBank (accession number, AP006627.1 and NC_002570, respectively) (http://www.ncbi.nlm.nih.gov) [13]. The nucleotide sequences of the promoter and the coding region of the *tnrA* belonging to *B. clausii EHY L2* deposited previously in GenBank were also used in this study (accession number, HM488959). Various TnrA protein sequences applied to this investigation are as follows: YP_175256 (*B. clausii KSM-K16*), HM488959 (*B. clausii EHY L2*), NP_242360 (*B. halodurans C-125*), YP_001813196 (*Exiguobacterium sibiricum 255-15*), YP_078674 (*B. licheniformis* ATCC 14580), NP_389214 (*B. subtilis subsp. subtilis str. 168*), YP_001420907 (*B. amyloliquefaciens FZB42*), YP_001486473 (*B. pumilus* SAFR-032), NP_691871 (*Oceanobacillus iheyensis* HTE831), YP_002886828 (*Exiguobacterium* sp. *AT1b*) and YP_002949574 (*Geobacillus* sp. WCH70).

Prediction of the TnrA boxes of *tnrA* and *glnA* promoter regions of *B. clausii* and *B. halodurans*

For prediction of the TnrA boxes of *tnrA* and *glnA* promoter regions of *B. clausii* and *B. halodurans*, the entire genomic nucleotide sequence of *B. clausii KSM-K16* and *B. halodurans C-125* obtained from GenBank were analyzed [13]. The 17-bp-long conserved DNA motif represented by the consensus sequence 5'-TGTNAN7TNACA-3' for the TnrA box, was then entered into the nucleotide basic local alignment search tool (blastn) at the NCBI site (http:// www.ncbi.nlm.nih.gov) [13] as a query sequence. If the putative TnrA binding site was located upstream of the translation start site, the gene (or corresponding operon) was assigned to the potentially TnrA regulon. Furthermore, pairwise alignments between the consensus sequence of the TnrA box and all the promoter sequences of *tnrA* and *glnA* genes which have been identified as a TnrA regulon in B. subtilis were performed using the ClustalW2 program (http://www.ebi.ac.uk/Tools/clustalw2/) [14]. For identification of the promoter position of the potentially TnrA regulated genes, i.e. the transcription start site (TSS) and -35 and -10 promoter elements, the bacterial promoter prediction program, BPROM (www.softberry.com/berry.html) was used. The BioCyc database collection, which is a set of biological databases (http://biocyc.org), was used for describing the genome and metabolic pathways. Finally, the national microbial pathogen data resource (NMPDR) (http://www.nmpdr.org) was used for comparative analysis of the *B. clausii* and *B. halodurans* genome with other bacteria. Protein homology searches were carried out by the Position-Specific Iterated (PSI)-BLAST program (http://www.ncbi.nlm.nih.gov)[15].

Secondary structure prediction

The PSIPRED protein structure prediction program (http://bioinf.cs.ucl.ac.uk/psipred) was used for predicting the secondary structure of the *B. clausii* and *B. halodurans* TnrA proteins [16]. Conserved and functional domains of the protein were identified by using reverse position specific BLAST (RPS-BLAST) (http://www.ncbi.nlm.nih.gov). The program COILS was used to predict the coiled-coil regions of the protein [17]).

Phylogenetic analysis of TnrA

The TnrA and GlnA sequences of *B. clausii* and *B. halodurans* were compared with its ortholog sequences in the NCBI database using BLAST and aligned using the molecular evolutionary genetics analysis (MEGA) software, version 4.0 [18]. Phylogenetic trees were subsequently constructed by the neighbor-joining (NJ) method.

Results

Nucleotide sequence of the *glnA* promoter regions of *B. clausii* and *B. halodurans*

A genome analysis for *glnA* promoter regions of *B. clausii* and *B. halodurans* associated with a TnrA box was performed. *Bacillus clausii* contains two paralogs of the gene encoding the GS, *glnA1 (ABC3940)* and *glnA2 (ABC2179).* The *glnA1* gene, whose product has 452 amino acids, contains a TnrA site, 87 bp upstream of the translation start site. This TnrA site is located downstream of the -10 region of the promoter (Figure 1). Comparison of the deduced amino acid sequences of this *B. clausii* GS with other bacteria revealed that the *B. halodurans* GS (BH3867) has a high degree of similarity (91%) with that of *B. clausii.* However, the *B. clausii glnA2 (ABC2179)* gene, whose product has 449 amino acids, does not contain the TnrA site at its regulatory region. In fact, the GlnA1 and GlnA2 proteins of *B. clausii* were found to have only 69% sequence similarity (Figure 2).

It was also observed that *B. halodurans*, as in *B. clausii*, possesses two paralogs of *glnA*, with TnrA sites. The *glnA1 (BH2360)* gene contains a TnrA site, which overlaps the -10 region of the *glnA1* promoter, and the *glnA2 (BH3867)* gene contains a TnrA site downstream of the -10 region of its promoter. Considering the position of the TnrA box of GS genes in *B. clausii* and *B. halodurans*, downstream or overlapping the -10 region of the promoter, TnrA could act as a repressor that blocks initiation and/or elongation during the transcription process.

Nucleotide sequence of the *tnrA* promoter regions of *B. clausii* and *B. halodurans*

Alignment of TnrA sites in the promoters was used to compare the *tnrA* promoter sequences in *B. clausii KSM-K16* and *EHY L2, B. halodurans* and *B. subtilis.* The *tnrA* gene of the two *B. clausii* strains, *KSM-K16* and *EHY L2*, contains two TnrA sites in its promoter region, with a 25 bp interspace. The TnrA site1 overlaps with the -10 region and TnrA site2 lies upstream of the -35 region of the *tnrA* promoter. Comparison of the *tnrA* promoter sequences in *B. clausii KSM-K16* and *EHY L2* revealed that the TnrA boxes of both strains are located in the same positions, have similar sequences and consist of -10 and -35 elements in their *tnrA* promoter regions. The *tnrA* gene of the *B. subtilis* contains two TnrA sites in its promoter region with a 26 bp interspace. The *tnrA* gene of the *B. halodurans*, contains a TnrA sites in its promoter region (Figure 3).

Structure and properties of the *B. clausii* and *B. halodurans* TnrA proteins

The *B. clausii* and *B. halodurans* TnrA proteins are smaller than most MerR family members. It contains 100 amino acids and two domains (Figure 4). A conserved N-terminal DNA binding domain is located between residues 5 and 76. Based on the crystal structures

glnA1(ABC3940)	CATCCAATTGGATGGTTTTGCCATTATTATCTGAATATTAGTGTGTCAGATAATCTGACA...***ATG***
B.clausii	
glnA1 (BH2360)	TTTGTTAGATTAATTACTATGTCAGATTATCTTACATATATTTGACAGATCGATACCCTT...***ATG***
B.halodurans	
glnA2 (BH3867)	ATTCACTCTTTTTTTATTCTATTGTAGATTACC…GTTTGTCAGATAATCTAACAAACCCA…***ATG***
B.halodurans	
glnRA B.subtilis	ATTTGATGTTAAGAATCCTTACATCGTATTTGACACATAATATAACATCACCTATAATGA...***ATG***

Figure 1: Comparison of the nucleotide sequences of the GS promoter regions in *B. clausii, B. halodurans* and *B. subtilis*. The proposed -35 and -10 promoter elements are underlined. The TnrA sites are shown in boxes. The translational start site is indicated by bold italic letters.

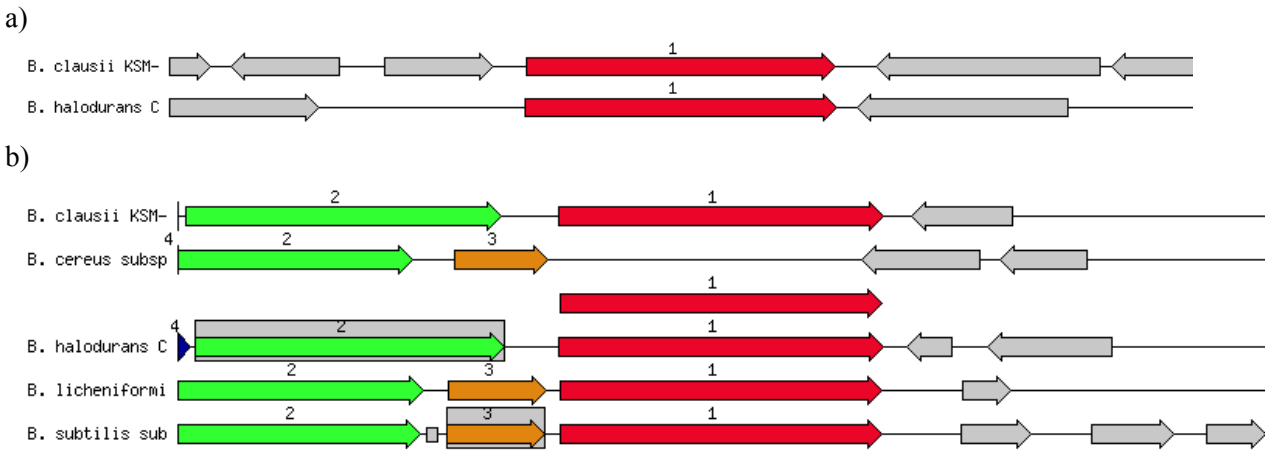

Figure 2: The chromosomal regions of the *B. clausii* and *B. halodurans glnA* are compared with other similar organisms. Sets of genes with similar sequence are grouped with the same number; a) The graphic is centered on the *B. clausii glnA1* (ABC3940) and *B. halodurans glnA2* (BH3867) genes, which numbered 1. b) The graphic is centered on the *B. clausii glnA2* (ABC2179), *B. halodurans glnA1* (BH2360) and *B. licheniformis and B. subtilis glnA* genes, which numbered 1, the number 2 is aluminum resistance protein and the number 3 is GlnR.

B. clausii KSM-K16	TTGTTAGATTTTCTTACATCAAAATGCACATCAATCGTTTTTGTGTGATATATTGTTACAAACAACATGGCGATTATCCATTACAGCGAGGTGGAAT...***GTG***
B. clausii EHY L2	TTGTCAGATTTTCTTACATTAAAATGCACATGAACCGTTTTTGTGTGATATATTGTTACAAAACAACATGGCTATTATCCGTTACAGTGAGGTGGAG...***GTG***
B. subtilis	TTGTTAATTTTTCTTCCGTATTTTTTCAAAAATCTTCTATTTGATGTTAGAAAATATGACAAGCGTCAGGTTTTTTCTCTTTGTTTTTTATATACTATT...***ATG***
B. halodurans	TTGTAAGAAAATCTAACATACCCTCTGTTCCTCTTGTTCCACTTATGCTATAGTAAAAATCAACAAAGGTCAGAACATTTTGAACAAGACGCTAAT…***GTG***

Figure 3: Comparison of the nucleotide sequence of the *tnrA* promoter region in *B. clausii KSM-K16, B. clausii EHY L2, B. subtilis* and *B. halodurans*. The proposed -35 and -10 promoter elements are underlined. The TnrA sites are shown in boxes. The translational start site is indicated by bold italic letters.

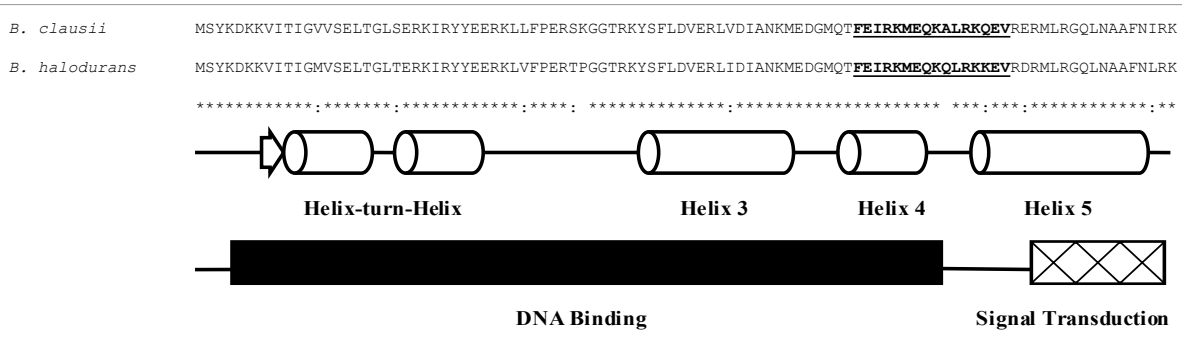

Figure 4: A graphical view of the secondary structure and domains of the *B. clausii* and *B. halodurans* TnrA proteins, where α-helices and β-strands are represented by cylinders and arrows. The coiled-coil domain is indicated by underlined bold letters and interface amino acids are underlined.

of the multidrug-binding transcription regulator BmrR of *B. subtilis* and secondary structure prediction by PSIPRED, this domain was shown to contain a β-strand, a helix-turn-helix motif formed by helices 1 and 2, and a second wing formed by helices 3 and 4. A conserved 15-amino-acid C-terminal region was also found, which like other TnrA orthologs, functions as a signal transduction domain. In fact a similar domain in the TnrA of *B. subtilis* has also been reported to be involved in signal transduction [19-21].

Using the COILS program [17], the C-terminal region of TnrA was predicted to contain coiled-coil structures, arising from the association of amino acid residues (68 to 83) with other similar C-terminal regions of the TnrA. Furthermore, Ile-70, Met-73, Ala-77 and Lys-80 were recognized as interface residues on α-helices 4 and 5 of the *B. clausii* TnrA protein (Figure 4).

Phylogenetic analysis of *tnrA* and *glnA* genes

Comparison of the deduced amino acid sequences showed that there was strong homology between the TnrA of *B. clausii* and *B. halodurans* (98% similarities at the amino acid levels). The generated phylogenetic tree showed that the TnrA sequences of *B. clausii* and *B. halodurans* were grouped together. Also, *Bacillus pumilus*, *B. subtilis*, *Bacillus amyloliquefaciens* and *B. licheniformis* fell into the same clade (Figure 5). Furthermore, the GlnA sequences of *B. clausii* (ABC2179) and *B. halodurans* (BH2360) were grouped together and *B. pumilus*, *B. subtilis*, *B. amyloliquefaciens* and *B. licheniformis* fell into the same clade (Figure 5).

It is important to note that the alkaline protease of *B. clausii,* similar to that of *B. subtilis*, is also expressed in abundance under nitrogen-limited conditions [10]. So it may be possible that the *aprE* (coding for alkaline protease) of *B. clausii* is also under nitrogen regulation through the GlnA-TnrA pathway. On this basis, a nitrogen-replete status in the cell may be a situation where TnrA is captured by complex formation with feedback-inhibited GlnA. Therefore, we propose to construct a potent *B. clausii* for the production of alkaline serine proteases by the disruption of *glnA* or truncation of the C-terminal region of *tnrA*,

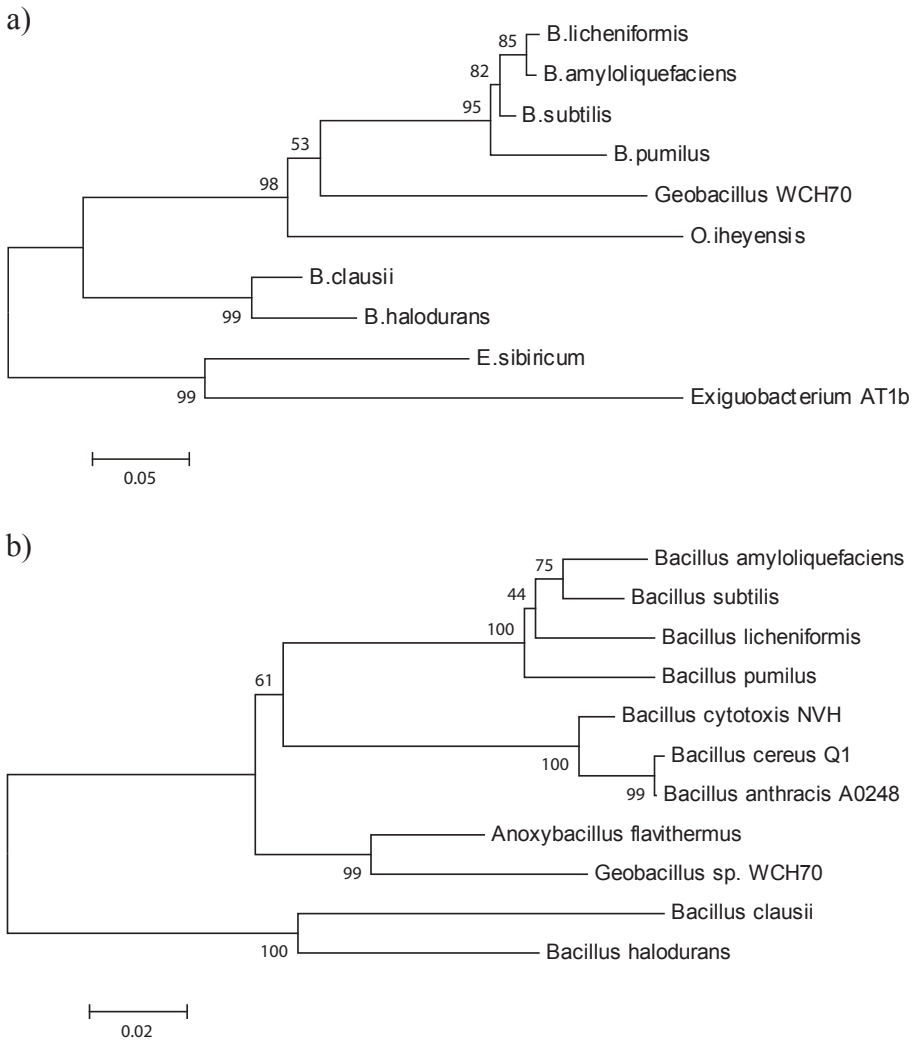

Figure 5: Phylogenetic trees of the TnrA (a) and GlnA (b) homologs from ***Bacillus*** species. Numbers above branches represent bootstrap values (1000 replicates) using the neighbor joining (NJ) method.

which will lead to the release of TnrA from the feedback-inhibited GlnA, thus mimicking a nitrogen limited situation.

Discussion

The transcription factor, TnrA, which is involved in the control of nitrogem metabolism is a monocistronic gene that together with its orthologs, has been reported in many *Bacillus* genomes and related genera, such as *B. clausii, B. halodurans, B. subtilis, B. licheniformis, O. iheyensis* and *G. kaustophilus*. However, by contrast *B. cereus* has been observed to lacks *tnrA* and only possesses the *glnR* gene [4]. Comparison of *tnrA* promoter sequences in *B. clausii, B. halodurans* and *B. subtilis* revealed that these bacteria have one or two sequences representing the TnrA Box in the promoter region of *tnrA*. The distance between the two *tnrA* box sites is approximately equal, containing 25 and 26 nucleotides in *B. clausii* and *B. subtilis*, respectively [22]. In future research, we propose to carry out experimental analysis of all *tnrA* promoters with regard to the location of the TnrA box in *Bacillus* strains that carry this transcriptional regulator.

Comparison of *tnrA* promoter sequences in *B. clausii* and *B. subtilis* reveals that the -10 and -35 regions have mismatches with the σ^A-consensus sequence (TATAAT and TTGACA). This suggests that the *tnrA* promoters are non optimal σ^A-dependent promoters with a low level of intrinsic transcriptional activity [22]. The TnrAs of *B. clausii, B. halodurans*, *Geobacillus* WCH70, *O. iheyensis*, *Exiguobacterium sibiricum* and *Exiguobacterium* AT1b contain 99-101 amino acids, while the TnrAs of *B. subtilis, B. amyloliquefaciens, B. licheniformis*, and *B. pumilus* contain 109-103 amino acids [3]. Comparison of the deduced amino acid sequences of *B. clausii* TnrA with other bacteria showed extensive similarity (98%) with *B. halodurans* and high degree (80%) with *B. subtilis*.

Bacillus clausii contains two paralogs of *glnA1* (ABC3940) and *glnA2* (ABC2179), encoding GS, of which only *glnA1* has the TnrA box**.** Also, *B. halodurans* contains two paralogs of *glnA1 (BH2360)* and *glnA2 (BH3867)*, both with TnrA box. This study proposes to carry out future experimental analysis of genes, *B. clausii* and *B. halodurans glnA1* and *glnA2,* in order to identify the enzyme involved in the formation of the GlnA-TnrA complex, which prevents TnrA from binding to DNA and that has a key role in nitrogen metabolism. *Bacillus halodurans* like *B. clausii* only has the *tnrA* and a monocistronic *glnA* operon, but is devoid of *glnR*. Glutamine synthetase is encoded by the dicistronic *glnRA* operon which contains TnrA site(s) in *B.subtilis, B. licheniformis, O. iheyensis* and *G. kaustophilus* [4,22].

Considering that the TnrA-binding site of the monocistronic *glnA* operon is preserved in both *B. clausii* and *B. halodurans*, it could be that in the *B. subtilis*, the TnrA-binding sites of the dicistronic *glnRA* operon play an important role in controlling the production of GS rather than that of the GlnR tanscription factor. In fact, in *B. subtilis*, GlnR is only involved in the regulation of its own operon (*glnRA*) and *tnrA* [22]. Hence, it may be possible that GlnR has a weak regulatory role in the nitrogen metabolism of *B. subtilis*. It proposed that the TnrA-regulated genes, *glnA, tnrA* and *nrgA,* play an important role in nitrogen metabolism of most bacilli [9].

Comparison of TnrA regulons, which contain the TnrA sites of *B. clausii* and *B. subtilis* revealed that the general transcription factor, TnrA (*tnrA*), the glutamine synthetase gene (*glnA*), oligopeptide ABC transporter operons, the assimilatory nitrate and nitrite reductase operon (*nas*), the genes of purine catabolism (*puc*) and ammonium transport (*nrg*A), are conserved in both *B. clausii* and *B. subtilis* bacteria [4,7,9].

References

1. Brown NL, Stoyanov JV, Kidd SP, Hobman JL (2003) The MerR family of transcriptional regulators. FEMS Microbiol 27: 145-163.
2. Wray LV Jr, Ferson AE, Rohrer K, Fisher SH (1996) TnrA, a transcription factor required for global nitrogen regulation in *Bacillus subtilis*. Proc Natl Acad Sci U S A 93: 8841-8845.
3. Wray LV Jr, Fisher SH (2007) Functional analysis of the carboxy-terminal region of *Bacillus subtilis* TnrA, a MerR family protein. J Bacteriol 189: 20-27.
4. Doroshchuk NA, Gel'fand MS, Rodionov DA (2006) [Regulation of nitrogen metabolism in gram-positive bacteria]. Mol Biol 40: 919-926.
5. Belitsky BR, Wray LV Jr, Fisher SH, Bohannon DE, Sonenshein AL (2000) Role of TnrA in nitrogen source-dependent repression of *Bacillus subtilis* glutamate synthase gene expression. J Bacteriol 182: 5939-5947.
6. Wray LVJr, Zalieckas JM, Fisher SH (2000) Purification and in vitro activities of the *Bacillus subtilis* TnrA transcription factor. J Mol Biol 300: 29-40.
7. Yoshida K, Yamaguchi H, Kinehara M, Ohki YH, Nakaura Y, et al. (2003) Identification of additional TnrA-regulated genes of *Bacillus subtilis* associated with a TnrA box. Mol Microbiol 49: 157-165.
8. Fisher SH (1999) Regulation of nitrogen metabolism in *Bacillus subtilis*: vive la difference! Mol Microbiol 32: 223-232.
9. Farazmand A, Yakhchali B, Shariati P, Minuchehr Z, Ofoghi H (2011) *In silico* genome-wide screening for TnrA-regulated genes of *Bacillus clausii*. Iran J Biotechnol.
10. Abe S, Yasumura A, Tanaka T (2009) Regulation of Bacillus subtilis aprE expression by glnA through inhibition of scoC and sigma(D)-dependent degR expression. J Bacteriol 191: 3050-3058.
11. Ghaemi Oskouiea F, Tabandehb F, Yakhchali B, Eftekhara F (2007) Response surface optimization of medium composition for alkaline protease production by *Bacillus clausii*. Biochem Eng J 1: 37-42.
12. Saeki K, Ozaki K, Kobayashi T, Ito S (2007) Detergent alkaline proteases: enzymatic properties, genes, and crystal structures. J Biosci Bioeng 103: 501-508.
13. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2005) GenBank. Nucleic Acids Res 33: 34-38.
14. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673-4680.
15. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402.
16. McGuffin LJ, Bryson K, Jones DT (2000) The PSIPRED protein structure prediction server. Bioinformatics 16: 404-405.
17. Lupas A, Van Dyke M, Stock J (1991) Predicting coiled coils from protein sequences. Science 252: 1162-1164.
18. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.
19. Wray LVJr, Zalieckas JM, Fisher SH (2001) Bacillus subtilis glutamine synthetase controls gene expression through a protein-protein interaction with transcription factor TnrA. Cell 107: 427-435.
20. Jones DT (1999) Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol 292: 195-202.
21. Newberry KJ, Huffman JL, Miller MC, Vazquez-Laslop N, Neyfakh AA, et al. (2008) Structures of BmrR-drug complexes reveal a rigid multidrug binding pocket and transcription activation through tyrosine expulsion. J biol chem 283: 26795-26804.
22. Zalieckas JM, Wray LV Jr, Fisher SH (2006) Cross-regulation of the Bacillus subtilis glnRA and tnrA genes provides evidence for DNA binding site discrimination by GlnR and TnrA. J Bacteriol 188: 2578-2585.