

## Short Communication

# In silico genome-wide screening for TnrA-regulated genes of *Bacillus clausii*

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### Abstract

*Bacillus clausii* TnrA transcription factor is required for global nitrogen regulation. In order to obtain an overview of gene regulation by TnrA in *B. clausii* KSM-K16, the entire genome of *B. clausii* was screened for the consensus sequence, 5'-TGTNAN7TNACA-3' known as the TnrA box, and 13 transcription units were found containing a putative TnrA box. The TnrA targets identified in this study were *tnrA*, *glnA*, *nrgA*, *nasFDEB*, *puc* genes, *licT*, the two operons of the oligopeptide ABC transporter, *lytR*, transcriptional regulator of the Lrp/AsnC family, sodium-dependent transporter of SNF family, *hyu* genes and a biochemically uncharacterized protein.

**Keywords:** Gene regulation; TnrA; *Bacillus clausii*; nitrogen metabolism

The MerR family is a group of transcriptional activators, which regulates gene expression and controls transcription in response to diverse physiological signals, such as nitrogen availability. The *B. clausii* TnrA transcription factor, a member of the MerR family has 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. When nitrogen sources are in excess, the 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 (Wray *et al.*, 2000).

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 halodurans* lacks GlnR but possesses a single TnrA regulator of nitrogen assimilation and two paralogs of *glnA*, both with TnrA-binding sites.

*Bacillus clausii* is known to produce a commercially important extracellular alkaline serine protease (AprE). This enzyme, produced during the stationary phase of growth, is temporally controlled and subjected to regulation by a large number of positive and negative regulators that inevitably allow for the effective use of the enzyme in the cell's surrounding environment (Ghaemi Oskouiea *et al.*, 2008; Saeki *et al.*, 2007). Elucidating of the molecular mechanisms of the metabolism and gene regulatory networks could thus be used to design metabolic engineering strategies for maximizing alkaline serine protease production in *B. clausii*.

The aim of this work was to distinguish and analyze the potentially TnrA regulons of *B. clausii*,

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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 (Newberry *et al.*, 2008; Wray *et al.*, 2001; Jones, 1999).

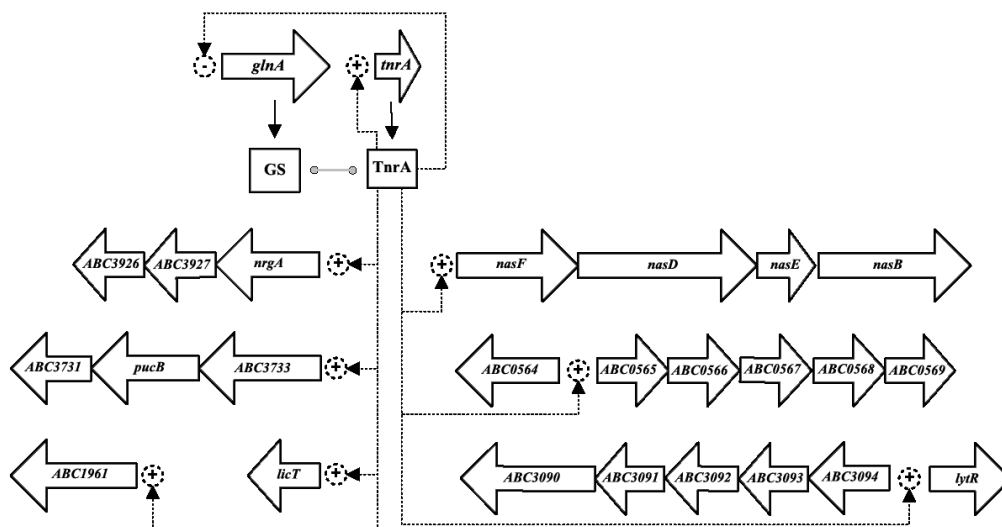
Using the COILS program (Lupas *et al.*, 1991), the C-terminal region of TnrA was predicted to contain coiled-coil structures, arising from the association of amino acid residues (68 to 83) with other similar C-terminal regions of the TnrA. Furthermore, Ile-70, Met-73, Ala-77 and Lys-80 were recognized as interface residues on  $\alpha$ -helices 4 and 5 of the TnrA protein (Fig. 2).

Comparison of the nucleotide and deduced amino acid sequences showed that there is strong homology between the *tnrA* genes of *B. clausii* KSM-K16 and *B.*

*clausii* *EHY L2* (97% and 100% identities at the nucleotide and amino acid levels, respectively). BLAST analysis was used to detect paralogous genes (Altschul *et al.*, 1997). *B. clausii* contains two paralogs of the gene encoding the GS, GlnA1 (*ABC3940*) and GlnA2 (*ABC2179*). The *glnA1* gene, 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. Comparison of the deduced amino acid sequences of the *B. clausii* GS with other bacteria revealed that the *B. halodurans* GS has a high degree of similarity (90%) with that of *B. clausii*. However, the *glnA2* gene does not contain the TnrA site at its regulatory region. *B. halodurans* like *B. clausii* only has the *tnrA* and a monocistronic *glnA* operon, but is devoid of *glnR*. *B. halodurans* possesses two paralogs of *glnA*, both with TnrA-binding sites (Fig. 3) (Doroshchuk *et al.*, 2006).

<i>glnA1 B. clausii</i>	CATCCAATTGGATGGTTTTGCCATTATTATCTGAATATTAGTG <b>GTGCAGATAATCTGACA</b> ...ATG
<i>glnA (BH2360) B. halodurans</i>	TTTGTTAGATTAATTACTA <b>GTGCAGATTATCTTACA</b> TATATTGACAGATCGATACCCCT...ATG
<i>glnA (BH3867) B. halodurans</i>	ATTCACTCTTTTTTATTCTATTGTAGATTACC...GTT <b>GTGCAGATAATCTAACA</b> AACCCA...ATG
<i>glnRA B. subtilis</i>	ATTTGAT <b>GTGTAAGAATCCTTACA</b> TCGTAT <b>TGACACATAATATAACA</b> TCACCTATAATGA...ATG

**Figure 3.** Comparison of the nucleotide sequences of the GS promoter regions in *B. clausii* KSM-K16, *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 4.** Regulatory network of nitrogen metabolism in *B. clausii*. Arrows indicate the relative length and organization of genes on the chromosome. Dotted black arrows illustrate the regulatory interactions of the respective regulator of nitrogen metabolism including its predicted function, in activating (plus) or repressing (minus) the target gene(s). Gray lines with circles indicate post-translational interactions.

The *nrgA* gene, whose product is an ammonium transporter, is present in the genome of *B. clausii* and has a conserved TnrA site in its regulatory region (Fig. 4). Similarly, the *nrgA* (*amtB*) of many other bacteria such as *B. subtilis*, *B. cereus*, *B. licheniformis*, *Oceanobacillus. iheyensis* and *G. Kaustophilus* contain one binding site for TnrA (Doroshchuk *et al.*, 2006). Bioinformatics analysis showed another set of genes associated with the TnrA box, known as the *nas* genes, which are involved in nitrate and nitrite assimilation, and are organized as two transcription units, *nasFDEB* and *nasCA*. The *nas* operon with the conserved TnrA site, occurs in the genomes of *B. clausii*, *B. subtilis*, *B. licheniformis*, *B. cereus* and *B. halodurans* (Doroshchuk *et al.*, 2006). The *puc* genes involved in purine catabolism contain a TnrA site. In *B. subtilis*, the *puc* operon contains two binding sites for TnrA (Fig. 4).

This study also identified the hydantoin utilization operon (*hyu*), as a new member of the potentially TnrA regulons, that contains a reliable TnrA site in the regulatory region of its promoter. The hydantoin operon, which among the *Bacillus* species appears to be unique to *B. clausii*, consists of genes encoding aspartate/glutamate hydantoin racemase; it is a hypothetical conserved protein, which is thought to be involved in hydantoin and pyrimidine utilization under nitrogen-limited conditions. This research also identified two other operons with TnrA sites, which encode the oligopeptide ABC transporter in *B. clausii*. It has already been shown that orthologs of these genes (*opp ABCDF*) found in *B. subtilis* and *B. licheniformis* are under the control of TnrA (Yoshida *et al.*, 2003; Doroshchuk *et al.*, 2006). The *licT* gene, whose product is a  $\beta$ -glucoside *bgl* operon transcriptional antiterminator protein of the BglG family, is also present in the genome of *B. clausii* and has a conserved TnrA site in its regulatory region. However, the ortholog of this gene is not regulated by TnrA in *B. subtilis* (Doroshchuk *et al.*, 2006; Yoshida *et al.*, 2003).

Hence, *B. clausii* TnrA responds by regulating not only genes for nitrogen utilization but also those involved in amino acid (*ABC3095*) and carbon (*licT*) metabolism (Lindner *et al.*, 2002). The TnrA-regulated gene *lytR*, a transcriptional attenuator of autolytic activity, was found only in *B. clausii*. By contrast the *B. subtilis* *LytR*, described as a transcriptional attenuator of itself and the *lytABC* operon, is not regulated by TnrA (Doroshchuk *et al.*, 2006; Yoshida *et al.*, 2003). Hence, it could be that in *B. clausii*, the TnrA regulator is involved indirectly in the inhibition of autolysis

under nitrogen-limited conditions. The *B. clausii* *ABC3095* gene of the Lrp/AsnC family of transcriptional regulators also has a potential TnrA site. The ortholog of this gene has been found not to be regulated by TnrA in other bacilli (Doroshchuk *et al.*, 2006; Yoshida *et al.*, 2003). Members of the Lrp family are small DNA-binding proteins with molecular masses of approximately 15 kDa, which are important regulatory systems of amino acid metabolism and related processes (Brinkman *et al.*, 2003). The TnrA-regulated sodium-dependent transporter gene (SNF family) was found only in *B. clausii*. The ortholog of this gene is not regulated by TnrA in *B. halodurans*, *Bacillus Pseudofirmus* and *O. iheyensis* (Doroshchuk *et al.*, 2006).

It is worth noting that most of the *B. clausii* TnrA target genes are very likely involved in the utilization of nitrogen sources, such as ammonium (*nrgA*), nitrite, nitrate (*nasFDEB* and *nasA*) and oligopeptides, thus emphasizing the physiological role of TnrA regulation. Furthermore, the *B. clausii* TnrA may also respond by regulating genes involved in amino acid (*ABC3095*) and carbon (*licT*) metabolism (Fig. 4).

Comparison of potentially TnrA regulons in *B. clausii* with those which have been previously studied in *B. subtilis*, *B. licheniformis*, *B. halodurans*, *O. iheyensis* and *G. kaustophilus* reveals that in all these strains the TnrA sites of the *glnA*, *tnrA* and *nrgA* promoters are conserved (Doroshchuk *et al.*, 2006; Yoshida *et al.*, 2003). 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 (*nrgA*), are conserved in both *B. clausii* and *B. subtilis* bacteria (Doroshchuk *et al.*, 2006; Yoshida *et al.*, 2003).

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 (Abe *et al.*, 2009). 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*, which will lead to the release of TnrA from the feedback-inhibited GlnA, thus mimicking a nitrogen limited situation.

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