Nucleotide Sequence and Features of the *Bacillus licheniformis* gnt Operon

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Abstract

*Bacillus licheniformis* was able to utilize gluconate as the sole carbon source as efficiently as *Bacillus subtilis* did. Southern analysis indicated that *B. licheniformis* likely possesses only one gnt determinant. The nucleotide sequence (6278 bp) of the *B. licheniformis* DNA containing the gnt operon was determined, revealing the five complete open reading frames (ORF; genes). The putative product of the first gene, oug, did not show any significant homology to known proteins, but those of the second to fifth genes exhibited striking homology to the gntRKPZ genes of *B. subtilis*, respectively, indicating that they are the corresponding gnt genes of *B. licheniformis*. Not only is the organization of the gnt genes of these two Bacilli highly conserved, but so are the cis regulatory elements of their gnt operon. Sequence analysis of the upstream regions of these two gnt operons implied that a chromosome rearrangement in *B. subtilis* might have occurred immediately upstream of the gnt operon during evolution, causing it to diverge from a common ancestor into *B. licheniformis* and *B. subtilis*.

Key words: *Bacillus licheniformis*; *Bacillus subtilis*; gnt (gluconate) operon; chromosome rearrangement

1. Introduction

Various microorganisms are able to grow on gluconate as the sole carbon source. Among the many gluconate utilization systems of these microorganisms, only that of *Bacillus subtilis* has been extensively studied at the molecular level.1-4 The gnt operon involved in gluconate catabolism of *B. subtilis* (gntss) consists of four gnt genes, that is, gntRBs, gntKPs, gntPQs and gntZPs. The first gene (gntRBs) encodes the repressor protein of this gntBs operon.2,5 The second and third genes (gntKPs and gntPQs) encode gluconate kinase and permease, respectively; only these two enzymes are specifically involved in gluconate catabolism.1 The last gene (gntZBs) was predicted to encode 6-phosphogluconate dehydrogenase by homology search.6 The gntBs operon is transcribed as a polycistronic message initiating at the gntBs promoter upstream of the gntRBs gene and terminating downstream of the gntZBs gene.1 The GntRBs protein represses gntBs promoter-activated mRNA synthesis by binding to the gntBs operator near this promoter, and this repression is suppressed by gluconate or glucono-6-lactone.2,7 Furthermore, a cis sequence involved in catabolite repression of this operon was found to be located approximately 150 bp downstream of the gntBs promoter, that is, within the gntRBs gene.8

Since only the gnt operon from *B. subtilis* has been analyzed at the molecular level, the analysis of a gnt determinant of a *Bacillus* other than *B. subtilis* may identify common features of evolutionary diverged genes with analogous functions. In this communication, we describe cloning and sequencing of the gnt operon of *B. licheniformis* (gntB), suggesting that not only gene organization but also regulatory functions of the gnt operon are highly conserved between these two Bacilli.

1.1. Gluconate utilization and some regulatory aspects in *B. licheniformis*

*B. licheniformis* strain 5A2,9 which was supplied from Bacillus Genetic Stock Center (Ohio State University), was utilized in this study. This strain grew at 37°C in S6 medium10 containing 25 mM Na-gluconate as the sole carbon source with a doubling time of 1.2 h whereas *B. subtilis* strain 60015 grew under the same growth conditions with a doubling time of 1.4 h. This fact indicates that the gluconate utilization system of *B. licheniformis* is somewhat more efficient than that of *B. subtilis*. To investigate the regulatory functions of the gluconate utilization system of *B. licheniformis*, we measured the activity of gluconate kinase in *B. licheniformis* which had been grown in S6 medium containing casamino acids as the sole carbon source with and without gluconate and

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with gluconate and glucose. Table 1 shows that almost the same amount of gluconate kinase in B. licheniformis and B. subtilis as that in B. subtilis was induced by the addition of gluconate to the medium, and this induced synthesis was repressed to one-fourth under catabolite repression by simultaneous addition of glucose. Although catabolite repression of gluconate kinase synthesis of B. licheniformis was somewhat weak in comparison with that of B. subtilis (we do not know the reason for this leakiness), regulatory aspects of the gntB operon including its catabolite repression might be similar to those of the B. subtilis operon (trpC2 metCf).

Table 1. Gluconate kinase synthesis in B. licheniformis and B. subtilis

| Strain          | Gluconate kinase activity (nmol/min per mg) |
|-----------------|---------------------------------------------|
|                 | No Gnt | Gnt | Gnt+Glc |
| B. subtilis     | <1     | 38  | <1      |
| strain 60015a)  |        |     |         |
| B. licheniformis| <1     | 43  | 12      |
| strain 5A2      |        |     |         |

Table 2. Comparison of the gnt genes between B. licheniformis and B. subtilis

| GntR | GntK | GntP | GntZ |
|------|------|------|------|
| Codon numbera) |
| B. licheniformis | 243  | 513  | 448  | 467  |
| B. subtilis      | 243  | 513  | 448  | 468  |

Sequence homology (%)a)

| Amino acid | Nucleotide |
|------------|------------|
| 85         | 88         |
| 86         | 86         |
| 76         | 81         |
| 77         | 78         |

The codon number and sequence homology were obtained by analysis of the sequences with the GeneWorks 2.3 program (IntelliGenetics, Inc., California).

that B. licheniformis contains only one gnt determinant (data not shown). Figure 1 shows a 6278-bp sequence containing the gntB operon which contains five complete open reading frames (ORFs; genes). Transcription of all these genes was in the same direction (Figs. 1 and 2). The first complete ORF (147 amino acids) was designated as oug (Fig. 1). A homology search of the putative oug product to known proteins in the NBRF-PIR, Swiss Plot Protein Sequence Databases and a translated protein sequence database from NCBI-GenBank Nucleotide Sequence Database using the FASTA program did not reveal any significant homology (FASTA optimized score <74). However, the second to fifth genes exhibited very high homology to the gntR, gntK, gntP, and gntZ genes, respectively (Fig. 1 and Table 2), indicating that they are gnt counterparts of B. licheniformis operon (gntR, gntK, gntP, and gntZ). The fact that the gene order of the gntB operon is the same as that of the gntB operon suggests that the gntB operon is also under autoregulation by the GntRei protein. Moreover, we found the same 5-bp overlap between gntR and gntK (Fig. 1) as between gntR and gntK.

1.2. Organization of the gnt genes of B. licheniformis

Southern analysis with a DNA fragment carrying the coding region of the gntR gene as a probe suggested...
**B. licheniformis**

![Gene organization](image)

**B. subtilis**

Figure 2. Gene organization of the gnt regions of *B. licheniformis* and *B. subtilis*. The upstream region of the *B. subtilis* gnt operon was cloned by screening a *B. subtilis* genomic library of λDASH II with the EcoRI 2-kb fragment containing the upstream portion of the gntBs operon cloned in plasmid pGNT41 as a probe. The 12.6-kb insert of a recombinant clone BE2-5 obtained, which was located upstream of the gntBs operon, was systematically sequenced as described previously, resulting in a 12586-bp sequence. A 136-bp nucleotide sequence of the short gap between the nucleotide sequence of the gntB operon (5482 bp) and a newly determined sequence (12586 bp) was determined after preparing templates for sequencing by PCR using the chromosome DNA as described previously. Among the newly identified 13 complete ORFs, only the S14A gene is shown, which is closest to the *B. subtilis* gnt operon. Pgnt and Tgnt indicate the gnt promoter and terminator, respectively. The *B. subtilis* sequence (18204 bp) will appear in the GSDB, DDBJ, EMBL, NCBI Nucleotide Sequence Databases under accession No. D31629.

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1.3. cis-regulatory sequences of the gnt operon of *B. licheniformis*

In a *B. licheniformis* nucleotide sequence [888 to 1128 nucleotides (nt)] corresponding to the region containing all of the cis regulatory sequences found in the promoter region of the gntBs operon, nucleotides which are identical to those of the *B. subtilis* sequence are boxed (Fig. 1). Regions possessing identical sequences longer than 17 bp are numbered I to IV from the most upstream region (Fig. 1). Regions I and II contained the same “-35” and “-10” sequences as those of the gntP operon promoter; the latter also contains the same gnt operator sequence as that interacting with the GntRBS protein. Region III contains the same Shine-Dalgarno sequence and translation initiation codon (ATG) as those for gntRBS. Region IV contains the same cis sequence as that for catabolite repression of the gntBs operon. These facts suggest that the gnt operon of *B. licheniformis* might be under the same regulation as that of *B. subtilis*.

1.4. Chromosomal rearrangement immediately upstream of the *B. subtilis* gnt operon

As shown in Fig. 2, sequencing of the upstream region of the gntBs operon revealed an ORF (designated S14A). A homology search of the putative S14A protein revealed striking homology to an *Escherichia coli* putative 42.1-kDa protein (FASTA optimized score, 1076) which is encoded in ORF3 upstream of the *rnpB* gene. The direction of this S14A gene transcription is opposite to that of the gntBs operon whereas that of the oug gene upstream of the gntBs operon is the same as this operon (Fig. 2). Furthermore, our unpublished results (K. Yoshida and Y. Fujita) indicated that the direction of transcription of the gntBs operon is opposite to that of the movement of the replication fork. This fact is not consistent with the general observation that the *B. subtilis* genes near the replication origin, like the gntBs operon, are often transcribed in the same direction as the movement of the replication fork. High homology between the gntBs and gntRBS operons, which started from the 3' end of the reported sequence for gntBs, continued up to -77 nt in the upstream region.
of the gnt\(_{\text{BS}}\) operon [transcription initiation nucleotide, +1] and up to 887 nt of the \(B.\ \text{licheniformis}\) sequence (Fig. 1). Interestingly, we found a palindromic sequence [TACCATGCAATATGGTA] complementary bases are underlined; with an adenine at -78 nt (dotted) at its center] which is located between -70 to -86 nt in the gnt\(_{\text{BS}}\) sequence (Fig. 1).

When the \(B.\ \text{licheniformis}\) sequence from 1 to 887 nt was searched for homology to a non-redundant nucleic acid sequence database constructed from the GSDB, DDBJ, EMBL and NCBI Nucleotide Sequence Databases using the FASTA program,\(^16\) it exhibited significant homology only to the 3' extreme of the reported sequence for the \(B.\ \text{subtilis}\) sigB operon\(^{20}\) (FASTA optimized score=255, 49.3%/531 bp); this sigB gene is located at 39° whereas the gnt\(_{\text{BS}}\) operon is located at 344° in the genetic map of \(B.\ \text{subtilis}.\)^{21}

All these facts imply that the gnt\(_{\text{BS}}\) operon might have been translocated from the previous location close to the sigB gene to the current location by chromosome rearrangement during evolution, causing to diverge from a common ancestor into \(B.\ \text{subtilis}\) and \(B.\ \text{licheniformis}\). The above palindromic sequence centered at 78 bp upstream of the transcription initiation nucleotide of the gnt\(_{\text{BS}}\) operon might be a vestige of this rearrangement.

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References

1. Fujita, Y., Fujita, T., Miwa, Y., Nihashi, J., and Aratan, Y. 1986, Organization and transcription of the gluconate operon, gnt, of \(B.\ \text{subtilis}\), \(J.\ \text{Biol. Chem.}\), \textbf{261}, 13744–13753.

2. Miwa, Y. and Fujita, Y. 1988, Purification and characterization of a repressor for the \(B.\ \text{subtilis}\) gnt operon, \(J.\ \text{Biol. Chem.}\), \textbf{263}, 13252–13257.

3. Yoshida, K., Fujita, Y., and Sarai, A. 1993, Missense mutations in the \(B.\ \text{subtilis}\) gnt repressor that diminish operator binding ability, \(J.\ \text{Mol. Biol.}\), \textbf{231}, 167–174.

4. Fujita, Y. and Miwa, Y. 1994, Catabolite repression of the \(B.\ \text{subtilis}\) gnt operon mediated by the CcpA protein, \(J.\ \text{Bacteriol.}\), \textbf{176}, 511–513.

5. Fujita, Y. and Fujita, T. 1987, The gluconate operon gnt of \(B.\ \text{subtilis}\) encodes its own transcriptional negative regulator, \(Proc.\ \text{Nat. Acad. Sci. USA}\), \textbf{84}, 4524–4528.

6. Reizer, A., Deutscher, J., Saier, M. H., Jr., and Reizer, J. 1991, Analysis of the gluconate (gnt) operon of \(B.\ \text{subtilis}\), \(Mol. Microbiol.\), \textbf{5}, 1081–1089.

7. Fujita, Y. and Miwa, Y. 1989, Identification of an operator sequence for the \(B.\ \text{subtilis}\) gnt operon, \(J.\ \text{Biol. Chem.}\), \textbf{264}, 4201–4206.

8. Miwa, Y. and Fujita, Y. 1993, Promoter-independent catabolite repression of the \(B.\ \text{subtilis}\) gnt operon, \(J.\ \text{Biochem.}\), \textbf{113}, 665–671.

9. Tyeryar, F. J., Jr., Lawton, W. D., and MacQuillan, A. M. 1968, Sequential replication of the chromosome of \(B.\ \text{licheniformis}\), \(J.\ \text{Bacteriol.}\), \textbf{95}, 2062–2069.

10. Fujita, Y. and Freeze, E. 1981, Isolation and properties of a \(B.\ \text{subtilis}\) mutant unable to produce fructose-bisphosphatease, \(J.\ \text{Bacteriol.}\), \textbf{145}, 760–767.

11. Nihashi, J. and Fujita, Y. 1984, Catabolite repression of inositol dehydrogenase and gluconate kinase synthesis in \(B.\ \text{subtilis}\), \(Biochim. Biophys. Acta\), \textbf{798}, 88–95.

12. Yanisch-Perron, C., Vieira, J., and Messing, J. 1985, Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors, \(Gene\), \textbf{33}, 103–119.

13. Yoshida, K., Sano, H., Miwa, Y., Ogasawara, N. and Fujita, Y. 1994, Cloning and nucleotide sequencing of a 15 kb region of the \(B.\ \text{subtilis}\) genome containing the \(\text{tol}\) operon, \(Microbiology\), in press.

14. Triglia, T., Peterson, M. G., and Kemp, D. J. 1988, A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequences, \(Nucleic\ \text{Acids Res.}\), \textbf{16}, 8186.

15. Miwa, Y. and Fujita, Y. 1987, Efficient utilization and operation of the gluconate-inducible system of the promoter of the \(B.\ \text{subtilis}\) gnt operon in \(Escherichia\ \text{coli}\), \(J.\ \text{Bacteriol.}\), \textbf{169}, 5333–5335.

16. Pearson, W. R. and Lipman, D. J. 1988, Improved tools for biological sequence comparison, \(Proc.\ \text{Nat. Acad. Sci. USA}\), \textbf{85}, 2444–2448.

17. Fujita, Y. and Fujita, T. 1986, Identification and nucleotide sequence of the promoter region of the \(B.\ \text{subtilis}\) gluconate operon, \(Nucleic\ \text{Acids Res.}\), \textbf{14}, 1237–1252.

18. Komine, Y. and Inokuchi, H. 1991, Precise mapping of the rrpB gene encoding the RNA component of RNase P in \(E.\ \text{coli}\), \(J.\ \text{Bacteriol.}\), \textbf{173}, 1813–1816.

19. Zeigler, D. R. and Dean, D. H. 1990, Orientation of genes in the \(B.\ \text{subtilis}\) chromosome, \(Genetics\), \textbf{125}, 703–708.

20. Kalman, S., Duncan, M. L., Thomas, S. M., and Price, C. W. 1990, Similar organization of the sigB and spoIIA operons encoding alternate sigma factors of \(B.\ \text{subtilis}\) RNA polymerase, \(J.\ \text{Bacteriol.}\), \textbf{172}, 5575–5585.

21. Anagnostopoulos, C., Piggot, P. J., and Hoch, J. A. 1993, In: Sonenshein, A. L., Hoch, J. A., and Losick, R. (eds) \(B.\ \text{subtilis}\) and other Gram-positive bacteria: biochemistry, physiology, and molecular genetics. The genetic map of \(B.\ \text{subtilis}\), American Society for Microbiology, Washington, D.C. pp. 425–461.