Systematic study of gene expression and transcription organization in the *gntZ-ywaA* region of the *Bacillus subtilis* genome

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Within the framework of the international project 'The functional analysis of the *Bacillus subtilis* genome' in Japan and Europe, the gene expression and transcription organization of the *gntZ-ywaA* region (160 kb) of the *B. subtilis* genome has been systematically analysed. First, all unanalysed genes comprising more than 80 amino acids (125 genes) in this region were inactivated through integration of plasmid pMUTIN. No essential gene was found which could not be inactivated. All the integrants grew normally in both nutrient sporulation medium and glucose minimal medium. But an integrant in the *yxbG* gene exhibited an oligosporogenic phenotype in the nutrient sporulation medium. The synthesis of β -galactosidase was examined, as a reporter for expression of the inactivated genes, during growth and sporulation in the two media. The results indicated that 36% of the promoters were inactive when cells were grown in at least one of these two media. Furthermore, the transcription of the 119 genes in this region was analysed by Northern blotting, resulting in a transcription map. The results indicate that the *gntZ-ywaA* region contains at least 24 polycistronic operons, including several published ones. The operons newly found in this work are *yxaAB*, *yxaGH*, *yxaJKL*, *yxbBA-yxnB-asnH-yxaM*, *yxbCD*, *yxced*, *yxdJK*, *yxeFGH*, *yxeKLMNOPQ*, *yxeR-yxxB*, *hutPHUIGM*, *bglPH-yxiE*, *wapA-yxxG*, *yxiM-deaD*, *katB-yxiS*, *yxjCDEF*, *yxjJI* and *yxkF-mmsX*.

Keywords: *Bacillus subtilis*, genome, transcription, gene inactivation, gene expression

INTRODUCTION

Recently, tens of genomes of micro-organisms, including *Saccharomyces cerevisiae* (Goffeau *et al.*, 1997) and *Bacillus subtilis* (Kunst *et al.*, 1997), have been sequenced. Among the numerous identified genes, nearly half were found to be functionally unknown ones. Determining the functions of the unknown genes of these micro-organisms will be an important step for a more complete and comprehensive understanding of their cellular processes and metabolic pathways. Thus, international projects on functional genomics of genetically well characterized *S. cerevisiae* and *B. subtilis* have been started, in which many research groups are

participating. Very recently, great progress in the field of functional genomics of *S. cerevisiae* has been made by research groups in Europe and North America, with the production of deletion strains for more than 2000 unknown genes and the assaying of the phenotypes of 500 of them in parallel (Winzeler *et al.*, 1999). On the other hand, many groups participating in the Japanese and European project on the functional analysis of the *B. subtilis* genome, which was initiated in 1996, have already inactivated nearly 2000 of the unanalysed genes through plasmid integration and are currently assaying the phenotypes of the integrants constructed, according to the following strategy.

The first step in the common strategy adopted in the *B. subtilis* genome function project is that each participating group is responsible for the inactivation of the unanalysed genes in a certain region of the genome. This

Abbreviations: β -Gal, β -galactosidase; DSM, defined nutrient sporulation medium; MM, glucose minimal medium; SD, Shine–Dalgarno.

inactivation is being carried out through integrational mutagenesis of a pMUTIN series of plasmids carrying *lacZ* as a reporter gene, as illustrated in Fig. 1 (Vagner *et al.*, 1998). Segments of the target genes are cloned into one of the pMUTIN plasmids, and the genomic copy is inactivated through a single- or double-crossover event. The promoter activities of the inactivated genes are monitored by measuring β -galactosidase (β -Gal) activity in cells during growth and sporulation, in both a defined nutrient sporulation medium (DSM) (Schaeffer *et al.*, 1965) and a glucose minimal medium (MM). These two media were chosen for the β -Gal monitoring because the catabolic and anabolic pathways are predominantly utilized in the growth in the nutrient and minimal media, respectively. Furthermore, several groups are also carrying out Northern blotting analysis of the unanalysed genes in their assigned regions under the same growth conditions as those for the β -Gal monitoring, thereby constructing a transcription map of the region. These analyses are expected to provide valuable knowledge about the transcription organization, expression profiles and functions of the target genes. For the second step of the project, each of the laboratories has screened, or will screen, integrants constructed through easily conductable high-throughput protocols, mainly using plate tests.

We are responsible for the inactivation and expression analysis of the unanalysed genes in the *gntZ*–*ywaA* region (160 kb) of the *B. subtilis* genome, the sequence of which has been determined by us (Yoshida *et al.*, 1996). As the first communication from the groups participating in this project, we describe the approach used for the inactivation of 125 unanalysed genes in this region, and the monitoring of their expression during cell growth and sporulation in DSM and MM. The results of our analyses provide valuable information about the essentiality of the inactivated genes, and their expression patterns during growth and sporulation in these media. We also describe the approach used for the construction of a transcription map of this region.

METHODS

Construction of bacterial strains. Table 1 comprises a list of the *B. subtilis* strains through integrational mutagenesis of one of the pMUTIN plasmids into the chromosome of *B. subtilis* Marburg 168 *trpC2* (Anagnostopoulos & Spizizen, 1961), the standard strain for the international project. The standard strain and pMUTIN plasmids [pMUTIN1, pMUTIN2 and pMUTIN4 (Vagner *et al.*, 1998)] were obtained from Drs V. Vagner and S. D. Ehrlich (Institut National de la Recherche Agronomique, Jouy-en-Josas, France).

Inactivation of target genes by integrational mutagenesis through a single-crossover event, as illustrated in Fig. 1, was essentially carried out as described by Vagner *et al.* (1998). The details and minor modifications are provided on the web site for the Japanese *B. subtilis* genome function analysis project (<http://bacillus.genome.ad.jp>). Either the 5' portion of the ORF of interest or the 5' region covering the Shine–Dalgarno (SD) sequence and initiation codon (100–300 bp long) was amplified by PCR using the chromosomal

DNA of strain 168 *trpC2* as a template. The sequences of the primer pairs, which were designed to generate flanking *Hind*III and *Bgl*II sites, are also available from the web site. The resulting PCR products were cleaved with *Hind*III and *Bgl*II, and then ligated into the *Hind*III/*Bam*HI sites of pMUTIN2. The ligated DNAs were digested with *Bam*HI and *Bgl*II to avoid self-ligation and used for the transformation of *Escherichia coli* strain C600 (Sambrook *et al.*, 1989), ampicillin resistance was used as the marker. The resulting plasmids, after being checked for the correct structure, were used for the transformation (Fujita & Fujita, 1983) of *B. subtilis* strain 168 *trpC2* with erythromycin resistance as the marker. Correct integration of a single copy of the plasmid into the desired locus of the chromosome was confirmed by Southern blotting (Sambrook *et al.*, 1989) or endonuclease-digestion analysis of the PCR product of the integrated region, results of which are provided on the web site.

Small target genes comprising less than approximately 200 bp were inactivated through a double-crossover event, which resulted in the deletion of part of their coding regions. This deletional inactivation was carried out as follows. The 5' portion of a target gene, including the SD sequence and translation-initiation codon, plus the 3' portion containing the translation-termination codon were amplified separately by PCR using the chromosomal DNA as template. The sequences of the two primer sets, which were designed to generate flanking *Eco*RI and *Bgl*II, and *Hind*III and *Eco*RI sites, are available from the web site. Each PCR product was cleaved with either *Eco*RI and *Bgl*II or *Hind*III and *Eco*RI, and the resulting two fragments were ligated into the *Hind*III/*Bam*HI sites of pMUTIN2. The ligated DNA was digested with *Bam*HI and *Bgl*II to avoid self-ligation, and then used for the transformation of *E. coli* strain C600 as described above. The resulting plasmids were linearized through *Eco*RI digestion, and then used for the transformation of *B. subtilis* strain 168 *trpC2* as described above.

Cell growth conditions and β -Gal assay. *B. subtilis* cells with integrations in the desired target genes were grown on TBABG (tryptose blood agar containing 10 mM glucose) plates containing 0.3 μ g erythromycin ml⁻¹ at 30 °C overnight. The cells were inoculated into 50 ml DSM (Schaeffer *et al.*, 1965) or MM in 200 ml Erlenmeyer flasks at OD₆₀₀ 0.05, and incubated at 37 °C in a water bath shaker. The composition of the MM (pH 7.0) was as follows: 0.4% glucose, 0.2% glutamine, 50 μ g tryptophan ml⁻¹, 62 mM K₂HPO₄, 44 mM KH₂PO₄, 11.4 mM K₂SO₄, 3.4 mM sodium citrate, 0.8 mM MgSO₄, 50 μ M CaCl₂, 24 μ M FeCl₃, 13 μ M ZnCl₂, 3 μ M Na₂MoO₄, 2.5 μ M CuCl₂, 2.5 μ M CoCl₂ and 1.32 μ M MnSO₄.

During growth and sporulation in DSM or MM, 1 ml aliquots (OD₆₀₀ 0.1–5.0) were withdrawn at 30 min or 1 h intervals, and the cells were collected by centrifugation and stored at –20 °C. The cells were suspended in 500 μ l Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄ and 1 mM DTT) containing 10 μ g DNase I ml⁻¹ (Sigma) and 100 μ g lysozyme ml⁻¹ (Sigma), and incubated at 37 °C for 20 min. After centrifugation (27 000 g, 20 min), the supernatants were used for spectrophotometric β -Gal assays, as previously described by Atkinson *et al.* (1990). One unit of β -Gal activity was taken as the amount of the enzyme that produced 1 nmol *o*-nitrophenol min⁻¹ at 25 °C.

Northern blotting. *B. subtilis* strain 168 *trpC2* cells grown in DSM or MM (OD₆₀₀ $\times$ ml = 100–400) were harvested. RNA was then purified as described previously (Fujita *et al.*, 1998). Northern blotting was also performed essentially as described

Fig. 1. Mutagenesis of a target gene through integration of pMUTIN. The disruption of a target gene (*yyyA* in this example) in an operon by integration of pMUTIN through a single-crossover event is illustrated. Details of the experimental procedures are given in Methods. A portion of the 5' region of *yyyA* ('*yyyA*') was cloned into pMUTIN1 or pMUTIN2 in *E. coli* strain C600 using ampicillin resistance as a selection marker. The resulting plasmid was used for transformation of *B. subtilis* strain 168 *trpC2* using erythromycin resistance as a selection marker. The construction of the resulting integrant is shown. *yyyA* expression under the control of the *PyyyAB* promoter can be monitored as *lacZ* expression. The downstream gene (*yyyB*) under the control of the *Pspac* promoter can be expressed through the addition of IPTG to the medium to avoid the eventual polar effect of the mutation in the upstream gene. As described by Vagner *et al.* (1998), the target gene (*yyyA*) can be inactivated through the replacement of its promoter with the *Pspac* promoter. When a target gene was essential for cell growth or it was hard to disrupt with the above method, a short DNA stretch carrying the SD and initiation codon of the gene was cloned into pMUTIN4 containing the *Pspac* promoter. The resulting plasmid was used for transformation, with erythromycin resistance as the selection marker, in the presence of IPTG on the plates.

previously (Sambrook *et al.*, 1989; Yoshida *et al.*, 1997), using total RNA and the ^{32}P -labelled PCR product for the 5' portion of the ORF of the analysed gene as a double-strand-specific DNA probe; the primer sequences are provided on the web site.

RESULTS AND DISCUSSION

Inactivation of the unanalysed genes in the *gntZ-ywaA* genomic region

Of the 155 genes in the 160 kb *gntZ-ywaA* region of the *B. subtilis* genome (Yoshida *et al.*, 1996; Kunst *et al.*, 1997), we did not inactivate *gntPKR*, *htpG*, *iolSR*, *deoR*, *dra*, *nupC*, *pdp*, *hutGIUHP*, *bglP*, *bglH*, *wapA*, *licT*, *bglS* and *licRBCAH* because they had already been characterized and *yxzG* had no clear SD sequence. The *yxzJ*, *yxzL*, *yxzE*, *yxzF* and *yxzCDE* genes were too small to be inactivated by pMUTIN integration through a single-crossover event. Nevertheless, the inactivation of *yxzCDE* was achieved through double-crossover recombination. The resulting 125 integrants are listed in Table 1, and the inactivated genes are also indicated in

Fig. 2. The gene-inactivation studies indicated that there were no essential genes for growth under the conditions examined among the 125 inactivated genes.

Examination of β -Gal synthesis under the control of the promoters of the inactivated genes

Expression of the inactivated genes was investigated by monitoring the activity of β -Gal, which was used as the reporter, during growth and sporulation. Sporulation of the 125 integrants after 24 h incubation in DSM was also examined microscopically, which revealed that only one integrant, YXBGd, was oligosporogenic. The efficiency of spore formation (heat-resistant) of this integrant after 24 h incubation in DSM was 3 % that of the viable cells, while that of strain 168 *trpC2* was over 70 %.

Table 1 shows the expression levels of the 125 inactivated genes during growth and sporulation in DSM and MM. This table also shows the maximal β -Gal activities of the integrant cultures during growth and sporulation in liquid DSM and MM, together with their expression patterns. More detailed results of the moni-

Table 1. Expression levels of the 125 inactivated genes in the *gntZ-ywaA* region

The experiments were repeated at least twice until consistent results were obtained. Representative results are shown.

Mutant*	Maximal β -Gal activity† [U mg ⁻¹ (time, expression pattern)]		Mutant*	Maximal β -Gal activity† [U mg ⁻¹ (time, expression pattern)]	
	DSM	MM		DSM	MM
GNTZd	41 (2, C)	45 (6, C)	YXIDd	9 (4·5, C+LV)	47 (9, C+S)
YXAAAd	6 (1, C)	23 (8, LV)	YXXDp	57 (2, C)	157 (9, LV)
YXABd	N	6 (10, LV)	YXXEd	27 (0·5, C)	73 (8, S)
YXACd	N	4 (7·5, LV)	YXIEd	106 (0·5, C+LV)	487 (9, C+S)
YXADd	81 (2·5, C)	39 (9, C)	YXXFd	N	27 (9, C+LV)
YXNAd	N	N	YXXGp	164 (4, C+LV)	443 (9, LV)
YXAFd	14 (2, LV)	6 (7, C+LV)	YXIFd	10 (4·5, LV)	42 (9, LV)
YXAGd	N	N	YXZCd	9 (4·5, LV)	42 (9, LV)
YXAHd	N	N	YXIGd	9 (4·5, LV)	29 (9, LV)
YXAl	201 (0·5, C)	222 (7, LV)	YXIhd	7 (4·5, LV)	30 (9, LV)
YXAJd	19 (0·5, C)	43 (8, LV)	YXIId	N	6 (9, S)
YXAKd	31 (0·5, C)	69 (9, LV)	YXIjd	5 (4·5, C)	20 (9, S)
YXALd	67 (0·5, C)	178 (9, LV)	YXIKd	5 (4·5, C)	15 (9, LV)
YXAMd	29 (4, T0)	42 (9, C+S)	YXIMd	15 (7·5, T2)	3 (9, S)
ASNHd	475 (5·5, T0)	435 (3, C+S)	DEADd	5 (0·5, C)	8 (8, S)
YXNBd	1424 (9, T0)	468 (9, C+S)	YXIOd	N	5 (7, C+LV)
YXBAAd	1499 (9, T0)	677 (9, C+S)	YXIPd	5 (0·5, C)	53 (8, LV)
YXBBd	2710 (4·5, T0)	1891 (8, C+S)	YXIQd	29 (7, T2)	N
YXBCd	111 (5, T0)	116 (9, C+S)	KATBd	N	N
YXBDd	12 (4·5, T0)	44 (9, C+S)	YXISd	30 (0·5, C)	20 (9, LV+S)
ALDXd	N	N	YXITd	8 (9, C)	6 (9, S)
YXBFd	N	3 (8·5, LV)	YXJAd	16 (3, C)	20 (9, C+LV)
YXBGd	N	7 (3, C)	YXJBd	10 (2, LV)	17 (9, LV)
YXCAd	6 (0·5, C)	16 (3, C)	YXJCd	32 (6, T1)	N
YXCCd	28 (0·5, C)	6 (4, C)	YXJd	43 (6·5, T1)	N
YXCDd	7 (5, C)	9 (9, C+LV)	YXJEd	20 (7·5, T1)	N
YXCEd	6 (3·5, C)	12 (8, C+LV)	YXJFd	17 (5·5, T1)	N
IOLAd	47 (5·5, LV)	13 (8, LV)	YXJGd	5 (2, C)	109 (5, C+LV)
IOLBd	25 (4·5, LV)	10 (9, C)	YXJHd	6 (0·5, C)	71 (5, C+LV)
IOLCd	9 (5·5, LV)	5 (3, C)	YXJId	9 (4, LV)	12 (8, S)
IOLDd	13 (5·5, LV)	3 (9, C)	YXJJd	98 (4, LV)	82 (4, C)
IOLEd	29 (5·5, LV)	5 (9, S)	PEPTd	96 (1, C)	127 (9, C+LV)
IOLFd	715 (7, T1)	21 (3, C)	YXJLd	N	N
IOLGd	721 (5, T0)	8 (3, C)	YXJMp	N	N
IOLHd	2391 (3, LV)	58 (3, C)	YXJNd	6 (3, C)	6 (7, C)
IOLId	1339 (3, LV)	136 (3, C)	YXJOd	N	6 (3, C)
IOLJd	1096 (3, LV)	97 (3, C)	YXKAd	N	N
YXDJd	11 (3·5, C+LV)	6 (9, C+LV)	GALEd	38 (3, C)	27 (9, C+LV)
YXDKd	5 (1·5, C)	N	YXKCd	177 (4, LV)	389 (5, C+LV)
YXDLd	6 (5, LV)	3 (8, C)	YXKd	8 (1·5, C)	3 (7, LV)
YXDMd	N	3 (4, C)	ALDYd	N	N
YXEAd	N	N	YXKEd	9 (8·5, C+LV)	7 (9, LV)
YXEBd	30 (3·5, C+LV)	60 (8, C+S)	MSMXd	2550 (6, C+LV)	564 (3, C+LV)
YXECd	59 (2·5, LV)	65 (7, C)	YXKHd	8 (3·5, LV)	19 (5, C+LV)
YXEDd	9 (9·5, T3)	N	YXKId	6 (0·5, C)	7 (5, LV)
YXEEd	145 (9·5, T4)	7 (7, C)	YXKJd	N	3 (9, LV)
YXEFd	N	3 (9, LV)	CYDAd	6 (0·5, C)	5 (9, C+S)
YXEGd	N	5 (9, LV)	CYDBd	7 (0·5, C)	9 (9, C+S)
YXEHd	6 (2, LV)	14 (7, C+LV)	CYDCd	N	4 (9, C+S)
YXEId	N	5 (7, C+S)	CYDDd	N	3 (9, C+S)
YXEKd	13 (2·5, LV)	26 (7, C+LV)	YXKOd	N	8 (3, C)
YXELd	N	6 (7, LV)	YXLAd	N	3 (3, C)
YXEMp	7 (2·5, LV)	9 (7, C+LV)	SIGYd	N	N
YXENd	9 (3, LV)	13 (7, C+LV)	YXLcd	9 (9·5, C+T3)	7 (9, C+LV)
YXEOd	12 (1·5, LV)	8 (7, C+LV)	YXLdd	N	N
YXEPd	5 (2·5, LV)	11 (8, C+LV)	YXLEdd	N	N
YXEQd	8 (3·5, LV)	19 (8, C+LV)	YXLfd	10 (9·5, LV+T3)	5 (8, C)
YXERd	N	8 (8, C)	YXLGd	N	N
YXXBd	N	N	YXLHd	N	N
HUTMd	166 (6, T0)	3 (4, C)	KATXd	N	3 (4, C)
YXIAd	N	N	YXLJd	N	N
YXIBd	20 (6, C+T0)	78 (9, S)	YWAAd	22 (4, C+T0)	101 (9, LV)
YXICd	55 (0·5, C)	128 (9, S)			

Table 1. For footnote see facing page.

toring of β -Gal synthesis can be found on the web site (<http://bacillus.genome.ad.jp>).

Among the 125 genes whose expression was examined by monitoring of β -Gal synthesis, 80 genes were expressed in both DSM and MM, 7 in DSM only, 20 in MM only and 18 in neither medium (Table 1). Seventeen genes (*yxaI*, *asnH*, *yxnB*, *yxbABC*, *iolFGHIJ*, *yxwE*, *hutM*, *yxiE*, *yxxG*, *yxcC* and *msmX*) were strongly expressed in cells during growth and sporulation in DSM: the cells produced more than 100 U β -Gal (mg protein)⁻¹. Of these genes, *iolFGHJ*, *yxwE* and *hutM* were not expressed strongly in cells grown in MM. *yxaL*, *yxiC*, *yxxD*, *yxjG*, *pepT* and *ywaA* were expressed strongly in MM only. Table 1 also shows the expression patterns of the genes during growth and sporulation.

The expression profiles of the inactivated genes might not fully reflect their expression *in vivo*, because expression of the genes examined might be auto-regulated or an inducer of an operon comprising several genes might be synthesized through the enzyme reaction(s) catalysed by their gene product(s). Moreover, it was observed that different locations of integration during mutagenesis affected the expression of *lacZ* (Y. Fujita & N. Yanai, unpublished results). It appears that the stability of an artificial mRNA encoding β -Gal, whose synthesis starts from the promoter of the target gene, is sometimes affected by the location of the crossover.

Construction of a transcription map of the *gntZ*–*ywaA* region

We performed Northern analysis of 119 genes to construct a transcription map of the *gntZ*–*ywaA* region (Fig. 2). Total RNA was extracted from cells of strain 168 *trpC2* grown in either DSM or MM and used for Northern blotting. The hybridization results are provided on the web site (<http://bacillus.genome.ad.jp>).

Fig. 2 shows the transcripts that were detected on Northern blots for the genes between *gntZ* and *ywaA*, together with their sizes, the times at which the cells were harvested and the medium used. The transcripts are placed beneath the analysed genes on the assumption that they were synthesized from upstream of them. When the same size of transcript was detected with multiple DNA probes from successive genes, the transcripts are appropriately placed beneath them according to their size (Fig. 2). Of the 119 genes analysed, the

transcripts of 77 genes were detected in both DSM and MM, 19 were detected in DSM only and 4 were detected in MM only. The transcripts of 19 genes were not detected in either medium.

A comparison of Table 1 and Fig. 2 indicates that the results of Northern blotting did not often coincide with those of β -Gal measurement. Among 115 genes, the expression of which was analysed by both Northern blotting and β -Gal measurement, the expression of 11 was detected only on Northern blotting and that of 14 was detected only on β -Gal measurement. Moreover, more than 10 U β -Gal (mg protein)⁻¹ was synthesized by the integrants in nearly 30% of the instances where no transcript of the target genes was detected in DSM or MM. One explanation for this is that there are differences in stability between the mRNAs of different target genes. Sometimes, rRNAs (2.9 and 1.5 kb) can hamper the detection of specific mRNA. For five genes (*yxwA*, *yxjL*, *yxcM*, *yxcA* and *aldY*), we could not detect transcription by either β -gal measurement or Northern blotting (Table 1 and Fig. 2).

Northern experiments revealed the operon structure of this region. The 14 newly found polycistronic operons were as follows: *yxaAB*, *yxaGH*, *yxaJKL*, *yxbBA*–*yxnB*–*asnH*–*yxaM*, *yxbCD*, *yxcED*, *yxdJK*, *yxwFGH*, *yxwKLMNOPQ*, *yxwR*–*yxxB*, *yxiM*–*deaD*, *yxcDEF*, *yxiJl* and *yxcF*–*msmX*. Recently, we detected *cydABCD* operon expression in cells grown on either solid DSM and MM or in liquid NSMP medium (Fujita & Freese, 1981) supplemented with glucose, but not in liquid DSM containing glucose or in liquid MM (Fig. 2) (Winstedt *et al.*, 1998). As shown in Fig. 2, we also detected the following polycistronic transcripts: 11.5 kb for *iolABCDEF*, 4.5 kb for *deoR*–*dra*–*nupC*–*pdp*, 8.0 kb for *hutPHUIGM*, 4.0 kb for *bglPH*–*yxiE*, 8.0 kb for *wapA*–*yxxG*, and 2.5 kb for *katB*–*yxiS*. In addition, we found a 4.3 kb transcript covering *sigY*–*yxwCDEFG*. We can not explain at the moment why this transcript continued to be synthesized to the oppositely oriented *yxwH* gene without termination of its synthesis between *yxwG* and *yxwH*. Table 1 also shows that there are very similar expression patterns for the integrants of the genes corresponding to polycistronic operons, except for *yxaG*–*yxaH*, *yxwR*–*yxxB* and *katB*–*yxiS* (Table 1).

In conclusion, we emphasize that this systematic study of gene expression and organization has provided valuable information for comparison with the results of future microarray-based analysis.

* In the nomenclature for the mutants the four capital letters of the gene names were used; the lower-case letters d, p and dd indicate integrational disruption through a single-crossover event, promoter replacement through a single crossover and deletional replacement through a double crossover, respectively. Integrants, the target genes of which are cotranscribed with other genes, are shown in bold. The operon structures of these genes are shown in Fig. 2.

† β -Gal synthesis during growth and sporulation of the integrants in DSM and MM was monitored as described in the text. The actual data are obtainable from the web site (<http://bacillus.genome.ad.jp>). The maximal β -Gal activities of integrants during growth and sporulation are shown as U (mg protein)⁻¹, together with the corresponding incubation times (h) after inoculation and the *lacZ* expression patterns. The expression patterns of *lacZ* are classified as no synthesis (N), constitutive synthesis (C) and induction at the late vegetative (LV), sporulation (T0 to T4), or stationary (S) phases. The cells entered the sporulation phase in DSM 3.5 h after inoculation (T0), while cells entered the stationary phase in MM 7 h after inoculation.

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