Sequence of the *Bacillus subtilis* genome region in the vicinity of the *lev* operon reveals two new extracytoplasmic function RNA polymerase sigma factors SigV and SigZ

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Two regions with sizes 18900 and 25400 bp, which join previously known contigs containing *levRDEFG*, *aadK* and *blt* genes near 235° of the *Bacillus subtilis* chromosome, were sequenced. Among others, two genes, which encode proteins homologous to RNA polymerase σ -factors, were identified within this region. The gene products designated SigV and SigZ, show the highest homology with σ -factors encoded by the gene *carQ* of *Myxococcus xanthus* and *sigX* (formerly *orfX20*) of *B. subtilis*, correspondingly. All σ -factors which show statistically significant homology to SigV and SigZ, belong to the ECF (extracytoplasmic functions) subfamily. SigV and SigZ do not have N-terminal sequence which prevents such proteins from binding to DNA without RNA polymerase core enzyme.

Keywords: *Bacillus subtilis* genome sequencing, extra cytoplasmic function σ -factors

An approach for understanding the functions of unknown genes, discovered by systematic genome sequencing, can be based on studies of complex regulons. The genes in such regulons, which we define as those for which expression depends on a pleiotropic regulator, are supposed to be related by their biological function. Assigning a gene to a regulon would therefore allow one to restrict the number of biochemical tests required to establish the gene function. The prominent candidates for such pleiotropic regulators in bacteria are the two-component signal transduction systems and RNA polymerase σ -factors. These regulators are responsible for induction of expression of different sets of genes highly specialized in driving the cell to one or another type of stationary phase differentiation. At least seven two-component systems (Msadek *et al.*, 1993) and 11 σ -factors (Haldenwang, 1995; Lonetto *et al.*, 1994) were described for *Bacillus subtilis*, a bacteria with a well-developed ability for environmental adaptation. We present here the results of sequencing analysis of 18900 and 25400 bp regions which join and extend

two previously known contigs which include *blt* (*Subti-List* contig SL194–2, Moszer *et al.*, 1996; Mizuno *et al.*, 1996; Sorokin *et al.*, 1996; Capuano *et al.*, 1996), *aadK* (Ohmiya *et al.*, 1989) and *sacC* (Parro *et al.*, 1997) genes near 235° on the *B. subtilis* chromosome (Bolotin *et al.*, 1996). Special attention is brought to structural characterization of new σ -factor genes, which we designate *sigV* and *sigZ*.

The 18900 and 25400 bp areas, corresponding to physical coordinates 2715 to 2790 kb on the *B. subtilis* chromosome (Itaya, 1993) were amplified by Long Accurate PCR as described elsewhere (Bolotin *et al.*, 1996). Sequencing was performed by cloning of this DNA, randomized by *Sau*3A, *Alu*I or *Hae*III restriction enzymes in M13mp18 or mp19 vector. Gap closure and final sequence verification was performed by direct sequencing of PCR products corresponding to gaps using the ABI PRISM dye-terminator sequencing kit (Perkin Elmer). The final sequence, determined on both strands, was analysed for putative genes as described by Sorokin *et al.* (1993). The main results of analysis are presented in Table 1 and Fig. 1. In 44300 bp of the new sequence we detected 41 ORFs. For 29 of them homology was found with known proteins, from which 24 have

The GenBank accession numbers for the sequences reported in this paper are U93874, U93875 and U93876.

Table 1. ORF features

ORF*	GenBank entry	Endpoints† (bp)	Size (aa)	Translation start‡	PIR entry	Description	FASTA score	Percentage match (no. aa)
<i>yrhA</i>	U93874	61–984	308	tattAAGGAGGaggacatat ATG	s35094	Cysteine synthase (<i>Spinacia oleracea</i>)	635	44.8 % (306)
<i>yrhB</i>	U93874	986–2125	380	tatacGAAGGAGGcatctgat ATG	a49864	Cystathionine gamma-lyase (<i>Rattus norvegicus</i>)	865	49.7 % (372)
<i>yrhC</i>	U93874	2208–2438	77	tctggcAGGTGATtctt TTG		No homologies	< 80	
<i>yrhD</i>	U93874	2474–2956c	161	aaacgatAGGAGGAaaacga ATG		No homologies	< 85	
<i>yrhE</i>	U93874	2975–5917c	981	aaagaagatGGAGGggaat ATG	a24698	Formate dehydrogenase chain A (<i>Methanobacterium formicicum</i>)	1206	35.6 % (683)
<i>yrhF</i>	U93874	6233–6601	123	atcgGAAGGGGTaatcagc ATG		No homologies	< 80	
<i>yrhG</i>	U93874	6864–7664	267	agaagtaatGGAGGcggttttatt ATG	a42712	Formate dehydrogenase (<i>Methanobacterium formicicum</i>)	616	44.9 % (276)
<i>yrhH</i>	U93874	8707–9249	181	ccaaaagcAGGTGtttaaa ATG	s30722	Hypothetical protein o251 (<i>E. coli</i>)	136	25.8 % (124)
<i>yrhI</i>	U93874	9472–10056	195	aatcGGAGGTgtgcagattac ATG	a42116	Repressor for cytochrome P450BM-3 (<i>B. megaterium</i>)	452	40.1 % (192)
<i>yrhJ</i>	U93874	10072–13236	1055	agGAAAGGGagatgtta ATG	a34286	Cytochrome P450BM-3 (<i>B. megaterium</i>)	3475	58.3 % (1048)
<i>yrhK</i>	U93874	13480–13770c	97	ttgaatGGAGGGATgata ATG		No homologies	< 90	
<i>yrhL</i>	U93874	13904–15808c	635	ctattGGAGGaataccc ATG	b64007	Hypothetical protein HI0392 (<i>H. influenzae</i>)	305	24.1 % (245)
<i>yrhM</i>	U93874	15919–16776c	286	acGAAGGAGGatctttctta ATG		No homologies	< 100	
<i>sigV</i>	U93874	16776–17276c	167	aaAAAGGAGccctttattgca ATG	s45561	Hypothetical protein OrfX20 (<i>B. subtilis</i>)	179	28.3 % (159)
<i>yrhO</i>	U93874	17472–18299c	276	tttatattGGAGGatat ATG	s55412	CymJ protein (<i>Klebsiella oxytoca</i>)	248	22.1 % (244)
<i>yrhP</i>	U93874	18452–18874§	141	tttAAAGGGGGagagaaca ATG	f64115	Virulence-induced protein MlgA homologue (<i>H. influenzae</i>)	168	26.2 % (130)
<i>yrpG</i>	U93875	10542–11492c	317	tatttAGGGAGAacagga TTG	s16390	Auxin-induced protein (<i>Nicotiana tabacum</i>)	291	31.0 % (290)
<i>sigZ</i>	U93875	11657–12187	177	aaattatAGGAGGTttttcaa ATG	a49704	Transcription initiation factor AlgU (<i>P. aeruginosa</i>)	152	23.8 % (152)
<i>yrpE</i>	U93875	12319–13074	252	aaaAAAGGAGTGttgga ATG		No homologies	< 80	
<i>yrpD</i>	U93875	14238–14945c	236	gatAGGGGGaaataatg ATG		No homologies	< 80	
<i>yrpC</i>	U93875	15326–16123c	266	cAGAAAGcGTGAgaagtg ATG	jc4005	Glutamate racemase (<i>Lactobacillus brevis</i>)	383	33.5 % (239)
<i>yrpB</i>	U93875	16473–17516c	348	cttAAcgGGAGGattcggt ATG	s50891	2-Nitropropane dioxygenase (<i>Williopsis saturnus</i>)	260	27.0 % (371)
<i>aadK</i>	U93875	17895–18494§	200	tGAAAcGGAGGaacatgta ATG	xxbsg	Aminoglycoside 6-adenyltransferase (<i>B. subtilis</i>)	1059	100 % (200)
<i>aadK</i>	U93876	2–787§	262	tGAAAcGGAGGaacatgta ATG	xxbsg	Aminoglycoside 6-adenyltransferase (<i>B. subtilis</i>)	1422	100 % (261)
<i>yrdA</i>	U93876	1013–1516	168	tgaAAAGGAGGatrrtt GTG		No homologies	< 80	
<i>yrdB</i>	U93876	2326–2697	124	gggtgtttttatagAGGAG TTG		No homologies	< 80	

Table 1 (cont.)

ORF*	GenBank entry	Endpoints† (bp)	Size (aa)	Translation start‡	PIR entry	Description	FASTA score	Percentage match (no. aa)
<i>yrdC</i>	U93876	2926–3489	188	tacgtAGGAGGgtatctt GTG	s28969	N-Carbamoylsarcosine amidohydrolase (<i>Arthrobacter</i> sp.)	131	37.5 % (72)
<i>yrdD</i>	U93876	3588–3737	50	ctcagAGGAGGgcatca ATG		No homologies	<60	
<i>yrdE</i>	U93876	4130–5362	411	aaagatGGAGGtcgaatga ATG	b42606	Cytochrome P450CVIIB1 (<i>Saccharopolyspora erythraea</i>)	881	41.8 % (392)
<i>yrdF</i>	U93876	5634–5909	92	taatGGAGGgcatatgttcta ATG	s01373	Ribonuclease inhibitor (<i>B. amyloliquefaciens</i>)	248	49.4 % (81)
<i>yrdG</i>	U93876	6243–6716	158	tattGGGGGTtgtgaa ATG	s59993	Leucine-responsive regulator (<i>S. typhimurium</i>)	316	32.7 % (150)
<i>yrdH</i>	U93876	6729–7493	255	atgtAGGAGGTtgttgat TTG	f64041	Hypothetical protein HI1738 (<i>H. influenzae</i>)	757	49.8 % (235)
<i>yrdI</i>	U93876	7490–7822	111	aagctGAGGTttccgt ATG	e64041	Hypothetical protein HI1737 (<i>H. influenzae</i>)	397	53.7 % (108)
<i>yrdJ</i>	U93876	7987–9309	441	ttaatcGGAGGatata ATG	s60180	Branched-chain amino acid carrier BrnQ (<i>Lactobacillus delbrueckii</i>)	1029	41.7 % (427)
<i>yrdK</i>	U93876	9267–9584	106	tattgcAGGAGGaat ATG		No homologies	<80	
<i>yrdL</i>	U93876	9742–10632c	297	caactatGGAGGactag ATG	b53305	CO ₂ fixation transcription activator CbbR (<i>Rhodospirillum rubrum</i>)	328	27.1 % (288)
<i>yrdN</i>	U93876	10966–11355	130	tagAAGGAGGggatttt ATG		No homologies	<80	
<i>yrdO</i>	U93876	11642–12577	312	ggtAAGGAGGagttttt ATG	d33830	Cation efflux system membrane protein CzcD (<i>Alcaligenes eutrophus</i>)	497	44.3 % (192)
<i>yrdP</i>	U93876	12665–13702	346	aaattggcGGGGTGTaat GTG	s29125	Dimethylaniline monooxygenase (<i>Homo sapiens</i>)	127	26.8 % (157)
<i>yrdQ</i>	U93876	13825–14691c	289	ttaatGAAGGGGGgatcttc ATG	b53305	CO ₂ fixation transcription activator CbbR (<i>Rhodospirillum rubrum</i>)	322	28.7 % (247)
<i>yrdR</i>	U93876	14817–15782	322	aAAAGGAGGggaattca ATG	e64182	Cysteine synthase CysZ homologue (<i>H. influenzae</i>)	98	19.3 % (233)
<i>yrkA</i>	U93876	16206–17510	435	actaggtAAGGcaaatca TTG	h64142	Hypothetical protein HI0107 (<i>H. influenzae</i>)	424	24.0 % (420)

* Nomenclature for the putative ORFs is described by Sorokin *et al.* (1996).

† In the corresponding GenBank entry.

‡ Putative start codons are indicated by bold letters and the nucleotides complementary to 16S RNA are indicated by upper-case letters.

§ Partial sequence in this GenBank entry. The complete size of this ORF is 855 bp.

their biological function or protein activity identified either experimentally or by similarity to other known proteins. Five ORFs encoded homologues of hypothetical proteins and for 12 no homology was found. There is no obvious 'functional orientation' of this area of the *B. subtilis* genome. However, our attention was drawn to two ORFs, designated *sigV* and *sigZ*, encoding proteins which have similarity with RNA polymerase

σ -factors. Using amino acid sequences of different σ -factors of this subfamily, revealed by homology to SigV and SigZ, we screened SWISS-PROT and Protein Information Resource (PIR) databases to detect all homologous proteins. Nineteen proteins were detected, having in most cases 22–30 % identical amino acids over the whole length with a FASTA score between 100 and 200 (not shown). These parameters were used to assign

Fig. 1. ORF map of the area sequenced. Overlapping GenBank entries are shown by hatched arrows. Details of the ORFs are presented in Table 1. Known genes are indicated above the scale bar. Sequences with accession numbers U93874, U93875 and U93876 were determined in the course of the present work. ORF designations are in accordance with the *B. subtilis* genome sequencing project (Moszer *et al.*, 1996).

a protein to this subfamily. Several pairs of proteins have more than 45% identical amino acids. These are *Escherichia coli* Fecl and *Pseudomonas putida* PupI (45.6% over 158 aa), *Pseudomonas aeruginosa* PvdS and *Pseudomonas fluorescens* PbrA (89.8% over 177 aa). *P. aeruginosa* AlgU shares 62–66% of identical amino acids with three σ -factors, called RpoE, from *E. coli*, *Salmonella typhimurium* and *Haemophilus influenzae*. The last three have 73–76% identity. The encoded regulators appear to belong to a recently recognized subfamily of σ -factors involved in the regulation of extracytoplasmic functions (ECF, Lonetto *et al.*, 1994). Three other such genes in *B. subtilis* were identified by systematic sequencing of the genome: *sigX* (Sorokin *et al.*, 1993), *sigY* (Yoshida *et al.*, 1996) and *ybbL*

Fig. 2. Multiple alignment of known ECF σ -factors from *B. subtilis*. The highly conserved regions 2 and 4 are boxed. See text for the nomenclature of σ -factors and the relevant references.

(proposed to be designated *sigW*) (H. Liu and others, accession no. D84214)

Multiple alignment of these five σ -factors (Fig. 2) reveals that seven amino acid residues are conserved in all of them. It also indicates that σ^Z and σ^V , as well as σ^X , do not have the N-terminal sequence which prevents these proteins from binding to DNA without RNA polymerase core enzyme (Dombroski *et al.*, 1992, 1993). It is worth mentioning also that three ECF σ -factor genes in *B. subtilis* have in their vicinity a gene which can be co-transcribed or divergently transcribed, thus forming an operon or a divergon with the corresponding σ -factor gene. These are: *ypuN* for *sigX*, *yrhM* for *sigV* and *yrpG* for *sigZ*. It was demonstrated in several cases that ECF σ -factors are negatively regulated by the products of such genes in their vicinity (van Hove *et al.*, 1990; Koster *et al.*, 1994; Huang *et al.*, 1997). This may therefore be a common feature for these ECF σ -factors in *B. subtilis*.

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