The European *Bacillus subtilis* genome sequencing project: current status and accessibility of the data from a new World Wide Web site

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Sequencing of microbial and eukaryotic genomes has recently gained considerable impetus. In Europe, the concerted efforts of a number of sequencing networks has led to the completion of the yeast genome (Dujon, 1996; Johnston, 1996) and to progress in the sequencing of a plant genome, *Arabidopsis thaliana* (Goodman *et al.*, 1995), and a microbial genome, *Bacillus subtilis* (Devine, 1995; Harwood & Wipat, 1996; Kunst *et al.*, 1995). The genomes of *Haemophilus influenzae* and *Mycoplasma genitalium* have been sequenced and published in their entirety (Fleischmann *et al.*, 1995; Fraser *et al.*, 1995), while the genome sequencing of a number of other bacteria of medical, industrial or ecological interest, such as *Synechocystis*, *Mycobacterium*, *Synechococcus*, and several archaeobacteria, is in progress or has been completed (Koonin *et al.*, 1996). The resulting opportunities for whole-genome comparisons will provide valuable insights into bacterial cell physiology and genome evolution (Tatusov *et al.*, 1996).

The progress of the European and Japanese *B. subtilis* genome sequencing projects up to September 1996 is summarized in Fig. 1. In addition to data from the sequencing project, this figure also includes information from the genetic and physical maps (Biaudet *et al.*, 1996; Itaya, 1993). By agreement with the co-ordinator of the Japanese genome sequencing project, we have defined a new origin for the *B. subtilis* chromosome that is different from the *Sfi*I site proposed previously (Itaya, 1993). The new origin is located at the point of cleavage within an *Eco*RV site between the *rpmH* and *dnaA* genes (Fig. 2). This newly defined site has the advantage of coinciding with both the origin of replication and the origin of the genetic map, and will provide the basis for future physical mapping of any sequence feature of the *B. subtilis* genome.

The locations of the *Not*I sites and *Sfi*I sites of the previously published physical map have been renumbered according to this new origin (see legend to Fig. 1).

Sequences deposited up to September 1996 in the European *B. subtilis* DNA sequence database, *SubtiList* (Moszer *et al.*, 1995), are represented in Fig. 1 as black bars. To facilitate the interpretation of this physical map, the positions of landmark genetic markers have been indicated. Whenever a disagreement exists between the position of a gene on the genetic or the physical maps, the sequence data are used to assign the correct position. The current assignment of regions to participants of the genome sequencing project is also shown. Four additional participants (C. Bruschi, Italy; A. Düsterhöft, Germany; F. Foury, Belgium; M. Vandenbol, Belgium) have recently joined the project and will sequence part of the DNA regions assigned to the European groups.

Since its first release, *SubtiList* has been updated on a regular basis. In the most recent version (September 1996), *SubtiList* contains 3311 kb of non-redundant DNA sequence, 79 % of the 4188 kb chromosome, extending over 99 contigs. This includes contributions from the European and Japanese sequencing networks (1684 kb and 1195 kb, respectively).

The annotated sequence (June 1996) comprises 2563 protein-encoding genes, 78 tRNA genes, 28 rRNA genes and 3 other RNA genes. A function has been assigned to 992 (39 %) of the protein-encoding genes – with or without experimental evidence – while 1571 genes (61 %) encode products of unknown function and are consequently identified by the starting letter 'y'. Known gene products can be ascribed to 45 functional categories (Table 1), an original classification derived from a number

Fig. 1. Physical, genetic and sequence map of the *B. subtilis* genome. According to the new origin of the chromosome (see text), *NotI* and *SfiI* sites have been assigned new positions as compared to the original physical map: 32 kb has been subtracted for sites located before the 18N *NotI* fragment (position 451–571), and 9 kb (32 kb minus 23 kb) for sites located after the 18N *NotI* fragment, taking into account the 23 kb deletion found in the strain originally used for construction of the physical map. BE, Belgium; CH, Switzerland; DE, Germany; ES, Spain; FR, France; IE, Ireland; IT, Italy; JP, Japan; KR, Korea; NL, The Netherlands.

Table 1. Functional classification of the known gene products of *B. subtilis*

1 Cell envelope and cellular processes		363 (36 %)
1.1	Cell wall	46
1.2	Transport/binding proteins and lipoproteins	78
1.3	Sensors (signal transduction)	9
1.4	Membrane bioenergetics	
1.4.1	Electron transport chain	22
1.4.2	ATP synthase	9
1.5	Mobility and chemotaxis	40
1.6	Protein secretion	11
1.7	Cell division	11
1.8	Sporulation	100
1.9	Germination	15
1.10	Transformation/competence	22
2 Intermediary metabolism		299 (30 %)
2.1	Metabolism of carbohydrates, sugar alcohols and amino sugars	
2.1.1	Specific pathways	48
2.1.2	Main glycolytic pathways	12
2.1.3	Pyruvate dehydrogenase complex	4
2.1.4	TCA cycle	11
2.1.5	Fermentation	11
2.2	Metabolism of amino acids and related molecules	96
2.3	Metabolism of nucleotides and nucleic acids	53
2.4	Metabolism of lipids	11
2.5	Metabolism of coenzymes and prosthetic groups	52
2.6	Metabolism of phosphate	1
2.7	Metabolism of sulfur	0
3 Information pathways		208 (21 %)
3.1	DNA replication	12
3.2	DNA restriction/modification and repair	11
3.3	DNA recombination	7
3.4	DNA packaging and segregation	6
3.5	RNA synthesis	
3.5.1	Initiation	11
3.5.2	Regulation	70
3.5.3	Elongation	6
3.5.4	Termination	3
3.6	RNA modification	2
3.7	Protein synthesis	
3.7.1	Ribosomal proteins	47
3.7.2	Aminoacyl-tRNA synthetases	18
3.7.3	Initiation	4
3.7.4	Elongation	2
3.7.5	Termination	1
3.8	Protein modification	3
3.9	Protein folding	5
4 Other functions		66 (7 %)
4.1	Adaptation to atypical conditions	11
4.2	Detoxification	3
4.3	Antibiotic production	17
4.4	Drug/analogue sensitivity	6
4.5	Phage-related functions	29
4.6	Transposon and IS	0
5 Miscellaneous		56 (6 %)

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gcgtttacggttattcgggttggaatgtttttcatttatgacacctccctcgaggaata
rpmH <-- AsnProGlnPheThrArgLysMet
gctgttaagadagtccttacttatttatatttgcgttacctattcattgtcaacttcacta
gtgcttttatttcttgcaaccataataggataccataccttttcaactttcgaaacctta
ttttttatgattccttaattttacggaaaaaagacaaattcaacaatttgcctctaaat
EcoRV
cagcgaTGTTGGATATcttttttgcgtttttttatGATCCACAGaggTTATCGACAacatt
A
+1 of B. subtilis genome
ttcacattaccaaccccTGTTGGACAAGgttttttcaacaggttgctcgtttTGTTGGATAA
gattgtgacaaccattgcaagctctcgttttattttgttattatttgtgttttaactct
tgattactaatcctacctttctcttTTATCCACAaagTGTTGGATAAgttggtgattgatt
tcacacagcttggtgtagaaggTTGTTCCACAagttgtgaaatttgctgaaaagctatttat
ctactatattatatgtttttcaacatttaattgtgtacgaatggttaagcgccatttgcctt
tttttgtgtctataacagagaagacgcatttttaagaaaaggaggacgtgccgga
agatggaaaatatattagacctgtggaaccaagcccttgc
MetGluAsnIleLeuAspLeuTrpAsn --> dnaA

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Fig. 2. New origin of the *B. subtilis* chromosome. DnaA boxes are indicated with upper case letters.

of others previously defined (Riley, 1993; Selkov *et al.*, 1996; N. Ogasawara, personal communication).

The gene products of unknown function have been divided into three classes: (i) those which show similarities with proteins whose function is known; (ii) those which show similarities with other proteins of unknown function; and (iii) those which do not exhibit any significant similarity with proteins in public data banks. Among the 1571 gene products of unknown function, 545 (35 %) fall into class (i), either because they show similarities with proteins whose function has been precisely demonstrated, or because they clearly belong to large functional families of proteins (such as sensor kinases and response regulators of two-component signal-transduction systems, LysR transcriptional regulators, ABC transporters, etc.), thus providing useful assistance in the elucidation of their actual function. Among the remaining gene products of unknown function, 290 (18 %) belong to class (ii) and 736 (47 %) to class (iii). Together, these 1026 gene products whose role cannot be predicted represent 40 % of those currently available for *B. subtilis*. This is in line with observations made during the course of other genome sequencing projects (Dujon, 1996; Fleischmann *et al.*, 1995), emphasizing the large proportion of genes that classical approaches have neglected. A systematic gene functional analysis programme supported by the European Commission has been initiated to challenge this issue (contract BIO2-CT95-0278).

The *SubtiList* database is available as a Macintosh or Windows stand-alone application – 4th Dimension (4D) runtime – by anonymous ftp at the URL <ftp://ftp.pasteur.fr/directory/pub/GenomeDB/SubtiList>. A World Wide Web (WWW) server has been set up recently (S. Brouillet and others, unpublished), taking advantage of the implementation of *SubtiList* on a UNIX system using the Sybase relational database management system. This server makes use of the same type of interface as the 4D version of *SubtiList*. The user can therefore navigate between the various sections of the database (contigs, genes, proteins), establish logical links

between different types of information, and perform multicriteria searches. Moreover, hyper-text links facilitate the retrieval of relevant information on *B. subtilis* gene and protein sequences from the EMBL (EBI server), SwissProt and ENZYME data banks (ExpASY server). A number of sequence analysis tools, such as rapid protein sequence comparisons (BLAST, FASTA) and DNA or protein pattern searches, are also directly accessible for the analysis of *B. subtilis* sequence data. The *SubtiList* WWW server can be reached at the URL <http://www.pasteur.fr/Bio/SubtiList.html>

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