Nucleotide sequence and analysis of the *phoB*–*rrnE*–*groESL* region of the *Bacillus subtilis* chromosome

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A 36 kb sequence of the *phoB*–*rrnE*–*groESL* region of the *Bacillus subtilis* chromosome at around 55° has been determined. The sequenced region contains 36 ORFs including the *phoB* and *groESL* genes, and the whole *rrnE* operon. The *phoB* gene is transcribed in the direction opposite to that of chromosome replication, while most ORFs, including *groESL* and the *rrnE* operon, are transcribed in the same direction. Two newly identified tRNA genes upstream of the *rrnE* operon were those for Arg-tRNA and Gly-tRNA. The sequenced region contains an operon consisting of genes for degradation and uptake of mannan. The *rrnE* operon and its downstream ORFs are well conserved among *Mycoplasma genitalium*, *Haemophilus influenzae*, *Synechocystis* sp. and *Methanococcus jannaschii*. σ^H consensus sequences are present in the promoter regions of three ORFs, including *groESL*.

Keywords: *Bacillus subtilis*, genome sequencing, *phoB*, *rrnE*, *groESL*

INTRODUCTION

Large-scale nucleotide sequencing eventually will reveal the structure and organization of genes on the chromosomes of bacteria (Bult *et al.*, 1996; Fleischmann *et al.*, 1995; Fraser *et al.*, 1995) that have been estimated by classical genetic methods in only a limited number of organisms. A cooperative effort, by many laboratories of different countries, for sequencing the whole genome of *Bacillus subtilis* (Kunst *et al.*, 1995; Ogasawara *et al.*, 1995) is providing fundamental knowledge of the gene organization of the spore-forming bacterial cell. For example, sporulation of *B. subtilis* is characterized by asymmetric septation and represents one of the simplest systems of differentiation (Errington, 1994). It requires different kinds of cellular activities such as signal transduction, transcriptional regulation and protein secretion; these functions are governed by many genes on the chromosome. Knowledge of the organization of the whole genome will provide more information about genes involved in these processes.

In this communication we describe the gene organization of the *phoB*–*rrnE*–*groESL* region of the *B. subtilis* chromosome at around 55° based on the nucleotide sequence of DNA fragments derived from chromosomal fragments synthesized by long accurate PCR. This region has only a few essential genetic markers.

METHODS

DNA synthesis, random cloning and DNA sequencing. Two DNA fragments of about 15 kb and one fragment of 5 kb were synthesized by long accurate PCR (Takara LAPCR kit, Takara thermal cycler TP3000) with chromosomal DNA as template and synthetic primers. The chromosomal DNA was isolated from the cells of strain JH642 (*trpC2 pheA1*) (from Y. Kobayashi, Tokyo University of Agriculture and Technology, Japan) grown in L broth at 37 °C, by treating the cells with 1 mg lysozyme ml⁻¹ and 1% (w/v) SDS in the presence of 50 mM EDTA, followed by treatment with phenol and 20 µg RNase I ml⁻¹. The sequences of the custom-made primer pairs used were: 5' GGCATACAATTTTTC ACGTCTTAATGATCATCGG 3'/5' CTCTGATAAATC CGCGGCTTTTTTCCCCCA3'; 5' GACAGGATAGAATT CCCCACAAAGATGCCGG3'/5' GGAACAGGCTGAGT GACTTACCTCTAAACG3'; and 5' TGCTGTCTAAAT CCACATGG 3'/5' GACACCGCGAAGCATTGCGCG 3'. These sequences were derived from the partially determined sequences of the chromosomal *NotI* or *SfiI* linking

Abbreviation: SD, Shine–Dalgarno sequence.

The GSDB, DDBJ, EMBL, NCB accession number for the nucleotide sequence reported in this paper is D88802.

Table 1. ORF analysis of the *phoB-rrnE-groESL* region of the *B. subtilis* chromosome

ORF	Characteristics*	SD/initiation codon†
<i>ydgK</i>	(nt 1–863) <i>E. coli</i> bicyclomycin resistance protein; P28246 (694) transmembrane	
<i>ydhB</i>	(nt 2503–864; 259 aa) transmembrane	<u>AA</u> tGGAGGaaaaaatatg
<i>ydhC</i>	(nt 1889–2560; 259 aa) <i>B. subtilis</i> gluconate operon repressor; P10585 (209) DNA binding	<u>AA</u> AGaggtattatatatg
<i>ydhD</i>	(nt 2690–4006; 439 aa) <i>B. subtilis</i> <i>yaaH</i> hypothetical protein; P37534 (704)	<u>AA</u> tGGgGGtttaaatatg
<i>ydhE</i>	(nt 4155–5297; 381 aa) <i>S. lividans</i> glycosyl transferase; JS0636 (311)	<u>AG</u> GCcatgtg
<i>ydhF</i>	(nt 6103–5342; 254 aa) membrane protein	<u>AA</u> AGaataacaaggtg
<i>phoB</i>	(nt 7503–6118; 462 aa) <i>B. subtilis</i> alkaline phosphatase IIIA; P19405 secretory (Bookstein <i>et al.</i> , 1990)	<u>AG</u> GAGGTtatccagttg
<i>ydhG</i>	(nt 7829–8233; 135 aa)	<u>AG</u> GAGGgaacaacatg
<i>ydhH</i>	(nt 8809–8315; 165 aa) secretory	<u>AG</u> GAGtcttttatg
<i>ydhI</i>	(nt 9309–8830; 160 aa) <i>B. subtilis</i> <i>ysaR</i> hypothetical protein; P46328 (512)	<u>AG</u> GAGtgttatg
<i>ydhJ</i>	(nt 9392–10366; 325 aa)	<u>GAG</u> GTGAcggcatg
<i>ydhK</i>	(nt 10511–11125; 205 aa) membrane protein	<u>GG</u> gcATgttttcattg
<i>ydhL</i>	(nt 12421–11147; 425 aa) <i>S. lividans</i> chloramphenicol resistance protein; P31141 (492) transmembrane	cctcaataatatggtttg
<i>ydhM</i>	(nt 12640–12948; 103 aa) <i>B. subtilis</i> , cellobiose phosphotransferase system, <i>celA</i> ; P46318 (220)	<u>AG</u> GgGGgatagcagttg
<i>ydhN</i>	(nt 12951–13280; 110 aa) <i>B. subtilis</i> cellobiose phosphotransferase system <i>celC</i> ; P46319 (233)	<u>AA</u> tGGtgaaatcaataatg
<i>ydhO</i>	(nt 13302–14625; 442 aa) <i>B. subtilis</i> cellobiose phosphotransferase system <i>celB</i> ; P46317 (998) transmembrane	<u>GG</u> Ataggggtg
<i>ydhP</i>	(nt 14648–16042; 465 aa) <i>C. thermocellum</i> β -glucosidase; P26208 (985)	<u>AA</u> GGggagagagacttg
<i>ydhQ</i>	(nt 16188–16898; 254 aa) <i>K. aerogenes</i> , histidine utilization repressor; P12380 (199) DNA binding	<u>GG</u> AGGataaccaatg
<i>ydhR</i>	(nt 16930–17826; 299 aa) <i>S. mutans</i> fructokinase; Q07211 (665)	<u>AA</u> GGAGGccATCtgcatg
<i>ydhS</i>	(nt 17826–18770; 315 aa) <i>B. subtilis</i> mannose-6-phosphate isomerase; P39841 (1049)	<u>GG</u> AGGTGcgatcatg
<i>ydhT</i>	(nt 18792–19877; 362 aa) <i>Bacillus</i> sp. mannan endo-1,4- β -mannosidase; P16699 (1049) secretory	<u>GG</u> AGtTGcatttg
<i>ydhU</i>	(nt 20396–19944; 151 aa)	<u>AA</u> cGGgtcgtgggtg
Arg-tRNA	(nt 21128–21204) ACG anticodon	
Gly-tRNA	(nt 21218–21291) UCC anticodon	
<i>rrnE</i>		
16S rRNA	(nt 21452–23004)	
23S rRNA	(nt 23173–26100)	
5S rRNA	(nt 26156–26271)	
Met-tRNA	(nt 26286–26362) CAU anticodon (Rudner <i>et al.</i> , 1993)	
Asp-tRNA	(nt 26423–26989) GUC anticodon (Rudner <i>et al.</i> , 1993)	
<i>ydiA</i>	(nt 26602–27654; 351 aa) <i>H. influenzae</i> hypothetical protein; E64115 (424) (Rudner <i>et al.</i> , 1993)	<u>GG</u> Aacatcttg
<i>ydiB</i>	(nt 27672–28145; 158 aa) <i>E. coli</i> hypothetical protein; P31805 (267)	<u>GG</u> TGActcgtgtg
<i>ydiC</i>	(nt 28129–28815; 229 aa) <i>H. influenzae</i> hypothetical protein; P43990 (182)	<u>AG</u> GAGttaagtagacatg
<i>ydiD</i>	(nt 28828–29280; 151 aa) <i>H. influenzae</i> , ribosomal protein alanine acetyltransferase; P44305 (189)	<u>GG</u> TGATaggatg
<i>ydiE</i>	(nt 29276–30313; 346 aa) <i>P. haemolytica</i> o-sialoglycoprotein endopeptidase; P36175 (660) transmembrane	<u>GG</u> TGAcgataaatg
<i>ydiF</i>	(nt 32474–30549; 642 aa) <i>H. influenzae</i> hypothetical ABC transporter; P44808 (974)	<u>GAG</u> GTGAaataaatg
<i>ydiG</i>	(nt 32600–33109; 170 aa) <i>E. coli</i> <i>moaC</i> protein; P30747 (368)	<u>AA</u> GGAcgtttttaaatg
<i>ydiH</i>	(nt 33109–33753; 215 aa)	<u>GG</u> AGGacccaaaatg
<i>ydiI</i>	(nt 33778–33948; 57 aa) membrane protein	<u>GAA</u> AGGAGGagcccaaatg

Table 1. Cont.

ORF	Characteristics*	SD/initiation codon†
<i>ydiJ</i>	(nt 33958–34719; 254 aa) <i>B. subtilis</i> <i>ycbT</i> hypothetical protein; P42252 (519) transmembrane	<u>AGAAAG</u> aagatcagtaagttagg atg
<i>ydiK</i>	(nt 34951–34763; 63 aa) secretory	<u>GGAGG</u> attattttt atg
<i>ydiL</i>	(nt 35682–34951; 244 aa) transmembrane	<u>AAAGGAG</u> tacataccat ttg
<i>groES</i>	(nt 35879–36202; 108 aa) (Schmidt <i>et al.</i> , 1992; Tozawa <i>et al.</i> , 1992)	ctcttttagtgctga gtg
<i>groEL</i>	(nt 36252–36308) (Schmidt <i>et al.</i> , 1992; Tozawa <i>et al.</i> , 1992)	<u>GGAGG</u> tcttgttaaac atg

* The characteristics of the ORFs comprise: (the beginning and end nucleotide numbers and the size of the protein in aa); the homologous protein and its accession number (SWISS-PROT); the FASTA score in parentheses [≥ 182 ; by DDBJ Homology Search System (see Methods)]; protein features [by PSORT Prediction Program (see Methods)]; and reference.

† SD sequence in underlined upper case and initiation codon in bold.

fragments in pNEXT26 and pSOFT8 (Itaya & Tanaka, 1991), a λ DASHII clone (from the *B. subtilis* DNA library of J. Nakai, University of Osaka, Japan) located between these linking genes and from the sequence of *groESL* (Schmidt *et al.*, 1992; Tozawa *et al.*, 1992).

The resulting DNA fragments of about 15 kb and 5 kb were purified by agarose gel electrophoresis, partially digested with DNase I (Takara), and cloned into the M13mp19 (Takara) cloning vector as described elsewhere (Yoshida *et al.*, 1995). A sample of DNA (20 μ g) was digested with 0.0056 U DNase I at 37 °C for 4.5 min in 400 μ l reaction mixture (50 mM Tris/HCl pH 7.5, 10 mM MnCl₂, 100 μ g BSA ml⁻¹). PCR screening was used to select for clones with inserts of sufficient size (> 400 bp). These clones were selected randomly and subsequently sequenced. Some regions were difficult to clone into phage vectors. Two of these regions, one of them located between *ydhL* and *ydhM* and the other between *ydhU* and the 16S rRNA genes, were directly sequenced with DNAs synthesized with primers outside the regions. Several primers were prepared and used to sequence the DNA covering the gap region.

Sequencing was performed with a DNA sequencer (373A, Applied Biosystems) using a *Taq* Dye Primer and Dye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequences obtained were assembled into contigs using a personal computer with the AutoAssembler (Version 1.3, Applied Biosystems).

Computer analysis. The DNA sequences determined were analysed with GeneWorks (version 2.0, IntelliGenetics), DISC Homology Search (<http://www.dna.affrc.go.jp/htbin/prefasta>), PSORT Prediction (<http://psort.nibb.ac.jp/form.html>), DDBJ Homology Search System (http://crick.genes.nig.ac.jp:8080/fasta_blast/html/Welcome.html), OWL (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/OWL/OWL.html>), TIGER'S WWW Server (<http://www.tigr.org/>) and CyanoBase (<http://www.kazusa.or.jp/cyano/cyano.html>).

RESULTS AND DISCUSSION

DNA sequence of the *phoB*–*rrnE*–*groESL* region of the *B. subtilis* JH642 chromosome at around 55°

We first partially sequenced two DNA fragments, pNEXT26 and pSOFT8, described as linking clones

Fig. 1. ORFs and RNA genes in the *phoB*–*rrnE*–*groESL* region of the *B. subtilis* JH642 chromosome. Transcription is from left to right (upper boxes) and right to left (lower boxes). Direction of chromosome replication is from left to right. Thick bars indicate *NotI* and *SfiI* linking fragments in pNEXT26 and pSOFT8. The λ DASHII clone used for the partial sequencing is also indicated. Arrows indicate DNA fragments synthesized by PCR.

(Itaya & Tanaka, 1991) by the deletion method and the region between the above clones which was obtained as a hybridizable λ DASHII clone. Based on the results obtained, we synthesized, by long accurate PCR, two DNA fragments each about 15 kb long which covered the above region, and one short DNA fragment of 5 kb long covering the region between pSOFT8 and *groESL*, and sequenced shorter fragments derived from these three DNAs after random digestion with DNase I and subcloning in M13mp19.

In total, 36 kb of DNA was sequenced (Fig. 1). All parts were sequenced on both strands, except for some regions in the *rrnE* and *groESL* genes where only one strand was

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12211          ydhL
CTAAATCATT TCGGATCTGA GGAAGAATTC CCCCACAAT TAACTCAACC
AATCCGACTG CAATCGTAGA AGCTGCAACA GGAAACTTT GAAATTCATA
ACAAACTCCT TTACTTAAT GTTTTGATAA ATAAAAAAA TCCTGATTAC
AAAAATGTC ATAACAAAT TTTGTAATCA GGATTTTACG GTTCCTGGTA
GACACCTCA ACCATATTA TTGAGGTAT ACAAGTGATA ATAGCTATTT
AATTGATTCG TTTCCGTGA TGAGTGTACC ACATTATGAA TGAATCTTCC
ATAGAAAAAT TTATAAACG GTGAACCGGA AAAAAATATG ATTATATTTA
TTTATATAAA GTATAGACAT TTAAATATA ATGACTATAA TAATCAATGT
AAGCGTTTAA ATAACAGGGG GGATAGCAGT TGAATAAAT ATTACTCGCG
TGCAGTTCAG GAATGTCTAC CAGTTTATTG GTGACAAAA TGAAGGAATA
          ydhM          12710

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Fig. 2. DNA sequence of the promoter region of *ydhL* and *ydhM*. Underlined regions indicate DNA sequences determined from only one strand. Start codons are boxed.

sequenced and compared with those sequences already described (Rudner *et al.*, 1993; Schmidt *et al.*, 1992; Tozawa *et al.*, 1992). The region between the *ydhL* and *ydhM* genes, which are transcribed in opposite directions, could not be sequenced on both strands. The sequences, nt 12245–12335 and nt 12495–12627 could be determined from only one strand. The former sequence was obtained from the DNA template complementary to the sequence shown in Fig. 2, and the latter sequence from a template DNA, the sequence of which is shown in Fig. 2. Sequencing from the opposite strand resulted in the frequent occurrence of N (any of the four possible nucleotides), probably because of non-specific or fairly equal incorporation of ddNTP. DNAs showing an N-rich pattern have sequences that are the same as those of the 5' portion of the presumed messenger RNAs of the *ydhL* and *ydhM* genes. Template DNA may take on tertiary structures and cause N-rich sequences by blocking DNA synthesis at specific points.

ORF analysis of the sequenced region

ORFs beginning with an ATG, GTG or TTG start codon and comprising more than 100 codons, were searched. As shown in Fig. 1 and Table 1, there were 36 ORFs, including the *phoB* (Bookstein *et al.*, 1990) and *groES/groEL* (Schmidt *et al.*, 1992; Tozawa *et al.*, 1992) genes. Except for *ydhL* and *groES*, Shine–Dalgarno (SD) sequences were detectable in these genes. The SD sequence is the complementary sequence to the 3' end sequence (UCUUUCCUCCACUAG...) of the 16S rRNA of *B. subtilis*. Twenty one, eight and six ORFs, respectively, had an ATG, TTG or GTG start codon.

The *ydhB* gene encodes a putative transmembrane protein with unknown function and has a σ^H promoter consensus sequence AGGAN₁₆GAAT (Haldenwang, 1995).

The *ydhC*, *ydhD* and *ydhE* genes seem to form an operon whose controlling element might be the *ydhC* gene as it encodes a putative protein with a DNA

HTH Motif

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YdhC      ..GERINETQLAKSFGVSRSPIREAMRLLEKD..
GntR      ..GTILSENTIAAEFSVSRSPVREALKILASE..

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YdhQ      ..QPIPDEVSIAKEFNSSRMTMKRALDNLVAE..
HutC      ..DRIPSEAEIVAQFGFSRMTINRALRELTDE..

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Fig. 3. Comparison of helix-turn-helix (HTH) motifs of YdhC and YdhQ with those of GntR (*B. subtilis* gluconate operon repressor) and HutC (*Klebsiella aerogenes* histidine utilization repressor). HTH regions are underlined. Amino acids common to all sequences are shown in bold letters. Identical amino acids between YdhC and GntR, and between YdhQ and HutC are indicated by italic letters.

binding domain similar to that of the gluconate operon repressor (Fujita *et al.*, 1986) (Fig. 3).

The *ydhF* gene, which encodes a transmembrane protein, may form an operon with the alkaline phosphatase gene.

The *ydhH* gene presumably encodes a secretory protein with a signal sequence. *ydhI* has a homologue (*ysaR*) on the *B. subtilis* chromosome.

The region from *ydhM* to *ydhT* may constitute a large operon involved in degradation and incorporation of mannan, although we need the transcription analysis to determine whether or not these genes really constitute a single operon. This hypothesis is based on the observation that the first three ORFs (*ydhM*, *ydhN*, *ydhO*) encode proteins homologous to the proteins involved in the phosphotransferase system of sugar uptake (PTS), where *ydhO* encodes a transmembrane protein. Following this putative operon, four ORFs encoding proteins with high similarity to sugar assimilation proteins are present: glucosidase (*ydhP*), fructokinase (*ydhR*), mannose-6-phosphate isomerase (*ydhS*) and mannanase (*ydhT*). YdhT has a putative signal sequence; hence it is likely to be a secreted protein. This operon might have an internal repressor as *ydhQ* encodes a protein having a DNA binding domain similar to that of the histidine utilization operon repressor, HutC (Schwacha & Bender, 1990) (Fig. 3).

The region between *ydhU* and *rrnE* has no ORFs (having more than 30 codons) with similarity to any known protein.

Five ORFs from *ydiA* (Rudner *et al.*, 1993) to *ydiE*, may constitute a large operon involved in macromolecule degradation as the last of these ORFs (*ydiE*) encodes a protein homologous to the glycoprotease.

The *ydiK* and *ydiL* genes probably constitute an operon. YdiL is a transmembrane protein and YdiK has a signal sequence and hence must be secreted. *ydiK* has a σ^H consensus promoter sequence, AGGAN₁₅GAAT (Haldenwang, 1995).

groES has a σ^H consensus promoter sequence, AGGGA₁₆GAAT (Haldenwang, 1995).

Fig. 4. The *rrnB* and *rrnE* regions of the *B. subtilis* chromosome.

The *rrnE* region

The *rrnE* region consists of four tRNA genes and three rRNA genes. We compared the sequence of the *rrnE* region and *rrnB* region as the latter also carries a cluster of tRNA genes (Green *et al.*, 1985). In the *rrnE* region, two tRNAs, for Arg and Gly, precede the consecutive 16S, 23S and 5S rRNA genes, which are followed by two tRNA genes for Met and Asp (Rudner *et al.*, 1993) (Fig. 4). These four tRNA genes are all found in the tRNA cluster of the *rrnB* region, although in the latter case these genes are all located behind the rRNA genes.

The second tRNA gene of the *rrnE* region, Gly-tRNA has two mutations (nt 21267, A; nt 21277, T) compared to Gly-tRNA (G, C) of the *rrnB* operon. These mutations occur pairwise at two sites of the TΨC stem of tRNA. The other three tRNA genes of the *rrnE* region are identical to those of the *rrnB* operon.

DNA sequences of *rrnE* and *rrnB* are well conserved with only one or two mutations over 100 bases from the end of Gly-tRNA to the end of 5S rRNA, including the spacer regions (boxed in Fig. 4).

Genes conserved among five bacterial species

B. subtilis genome sequences were compared with those from four other bacterial species: *Synechocystis* sp., *Haemophilus influenzae*, *Methanococcus jannaschii* and *Mycoplasma genitalium*. Similarity searches of our ORFs and RNA genes with those of the other bacteria gave the results shown in Table 2, where *rrnE* (16S and 23S) and the genes located downstream of *rrnE* (especially *ydiE*, a glycoprotein protease gene) are well conserved. Whereas *groEL* is also well conserved, *groES* is not. The genes of this region may have been conserved during evolution although the gene organization seems not to be conserved.

ACKNOWLEDGEMENTS

We thank Roy H. Doi for critical reading of the manuscript and helpful comments. This work was supported by a Grant-in-Aid for Creative Basic Research on 'Human Genome Analysis' from the Ministry of Education, Science and Culture of Japan.

Table 2. Genes conserved among five bacterial species

Homology searches were carried out using the Tiger Database GRATA program (<http://www.tigr.org/tdb/mdb/hidb/hidb.html>, <http://www.tigr.org/tdb/mdb/mjdb/mjdb.html> and <http://www.tigr.org/tdb/mdb/mgdb/mgdb.html>) and the CyanoBase BLASTP program (<http://www.kazusa.or.jp/cyano/cyano.html>). Scores (> 100) indicate *B. subtilis* (Bs) homologues in *Haemophilus influenzae* (Hi), *Methanococcus jannaschii* (Mj), *Mycoplasma genitalium* (Mg) and *Synechocystis* sp. (Ss).

Bs	Homology scores			
	Hi	Mj	Mg	Ss
<i>ydgK</i>	677			114
<i>ydhB</i>	127			140
<i>ydhC</i>	106			
<i>ydhD</i>				
<i>ydhE</i>				180
<i>ydhF</i>				
<i>phoB</i>				
<i>ydhG</i>				
<i>ydhH</i>				
<i>ydhI</i>				
<i>ydhJ</i>		204	136	
<i>ydhK</i>				
<i>ydhL</i>	406	137	106	132
<i>ydhM</i>				
<i>ydhN</i>				
<i>ydhO</i>		120		
<i>ydhP</i>				
<i>ydhQ</i>				
<i>ydhR</i>				103
<i>ydhS</i>				
<i>ydhT</i>				
<i>ydhU</i>				
Arg-tRNA	232	151	268	
Gly-tRNA	191		244	274
16S rRNA	4329	2197	3918	2539
23S rRNA	7078	3527	7139	2763
5S rRNA	331	196	150	
Met-tRNA	340	226	268	146
Asp-tRNA	306		115	127
<i>ydiA</i>	388	173		164
<i>ydiB</i>	232			188
<i>ydiC</i>	172			111
<i>ydiD</i>	175	138		161
<i>ydiE</i>	686	353	438	295
<i>ydiF</i>	911	181	135	669
<i>ydiG</i>	365	235		147
<i>ydiH</i>				
<i>ydiI</i>				114
<i>ydiJ</i>				135
<i>ydiK</i>				
<i>ydiL</i>				108
<i>groES</i>	212			266
<i>groEL</i>	1430	245	1094	633

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Received 11 November 1996; revised 14 January 1997; accepted 17 January 1997.